HOST FINDING POTENTIAL
OF FIRST INSTAR NOCTUID LARVAE OF
DIFFERENT HOST BREADTH

BY

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1995

To Rosa S. Garcés, Varsovia, Joan, Francesca, and Amanda.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
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December, 1995

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Nearly all studies of host-finding in Lepidoptera
concern the adult female and there is almost no knowledge of
the role of newly hatched larvae in finding and selecting a
host. The present study of survival, motility, and host
plant attraction of newly hatched larvae of three species of
Noctuidae (Lepidoptera) of different host breadth, *Heliothis
subflexa* (Guenée) (monophagous), *Trichoplusia ni* (Hübner)
(conservatively polyphagous), and *Spodoptera exigua* (Hübner)
(polyphagous), allowed me to assess the potential of first
instar larvae for host finding.

The survival of starved larvae of the three species was
similar during the first hours, but differed as time of
starvation increased. *H. subflexa* had a higher percentage of
survivors after 28 hours and it was also the only species to

survive up to 40 hours. *T. ni* and *S. exigua* had no survivors after 36 hours.

On a horizontal arena, *H. subflexa* larvae traveled a mean of 214.1 ± 11.3 (SE) cm/h, while larvae of *T. ni* and *S exigua* traveled 232.3 ± 8.4 (SE) cm/h and 141.1 ± 10 (SE) cm/h, respectively. *H. subflexa* climbed a mean distance of 173.9 ± 5.2 (SE) cm/h, while *T. ni* larvae moved 152.2 ± 6.1 (SE) cm/h, and *S. exigua* 67.7 ± 4 (SE) cm/h on vertical glass tubes. The rates of movement over time of the three species, in general, decreased as time of starvation increased.

A comparison of the overall rates of turning during horizontal movement of the three species suggests that *H. subflexa* moved in a straighter fashion than *T. ni* and *S. exigua*.

Assays in a four-choice olfactometer demonstrated that *T. ni* and *S. exigua* first instar larvae were significantly attracted to odor from cultivated and wild host plants. In addition, *T. ni* larvae exhibited significantly higher attraction to host plant odor than to non-host plant odor in competitive tests. Larvae responded anemotactically and chemotactically during oriented movement. Thus, the larvae studied can survive long periods of starvation, move considerable distances and orient to host odor, indicating a significant potential for first instar larvae in host-finding by the species.

CHAPTER 1
LITERATURE REVIEW AND RESEARCH AIMS

During the last four decades a vast amount of research
has been devoted to understanding insect-plant relationships.
A considerable number of these investigations has been
directed to host-plant selection, that being the essential
mechanism by which the plant-insect association is determined
(McIndoo, 1919, 1926; Dethier, 1937; Ahmad, 1983; Hanson,
1983; Courtney and Kibota, 1990). However, host selection by
larvae, and specifically, by first instar Lepidoptera larvae
has not been considered. This research was conducted to
determine the potential of larvae to find and select host
plants.

Terminology

Insect behavior that leads to finding a host plant
involves three steps: 1) movement, 2) detection of cues
(noncontact) from plant, and 3) response to the cues by
changing locomotion patterns, so that the probability of
contact increases (Kennedy, 1978, 1986; Miller and Strickler,
1984). Movement can be at random (kinesis) or directed
(taxes) to the source. The latter may be in response to
contact (chemotaxis) and noncontact cues (anemotaxis).

The terms used in insect plant-relationships have been the source of much confusion. Miller and Strickler (1984) made a revision of the terms applied in insect-plant interactions. Although it is difficult to categorize certain behaviors in some cases (Kennedy, 1978), the concepts presented by Miller and Strickler are acceptable, given the problem of covering the wide array of insect behavior. The main sequence of host-finding and accepting processes is integrated with the terminology of chemicals eliciting insect responses proposed by Dethier et al. (1960).

Finding, as defined by Miller and Strickler (1984), includes the behavioral mechanisms ranging from pure chance events to purposeful search in order to establish and maintain proximity to the resource. Thus, the term "locates" describes only the purposeful search. The finding process necessitates the movement of the insect. Finding essentially will include the following processes: (1) finder moves not influenced by host cues, (2) finder contacts cues from host, and (3) finder responds to the noncontact sensory cues by adjusting locomotion to increase proximity to host (arrestment) (Miller and Strickler, 1984). In the locomotion process, the strategies of movement are "at random" (kinesis), "with changes in the rate of speed" (orthokinesis), and "with changes in the rate of turning" (klinokinesis). In addition, these movements can be regulated by chemicals (chemokinesis). The movements can be directed towards a source (taxes) with chemical mediation

(chemotaxis) or in response to airflow (anemotaxis) (Bell, 1984). These definitions of orientation mechanisms were proposed by Kühn (1919; in Bell, 1984), and Fraenkel and Gunn (1940). Due to inconsistencies noted in this classification system, Bell and Tobin (1982) have suggested describing the process of orientation, instead of classifying the behaviors in one of these categories.

The terms attractants, stimulants, repellents, deterrents, and inhibitors as applied to volatiles are defined by Kennedy (1977, 1978) and Dethier et al. (1960). It has been suggested that the use of these definitions may avoid confusion when describing behavioral reactions to chemicals (Städler, 1992).

Host selection behavior following host finding are examination and consumption of the host, in which contact and noncontact cues are used (Miller and Strickler, 1984).

The term "prefer" has been used to describe insect foraging behaviors, in which plants are consumed more than expected based on their relative abundance. Thus, those plants would be preferred (Hassell and Southwood, 1978) and are often said to be selected over those less-preferred plants (Miller and Strickler, 1984). Although these terms have been recognized as simplistic in describing insect behaviors (Hassell and Southwood, 1978; Miller and Strickler, 1984), misconceptions can be avoided by using them correctly. Miller and Strickler (1984) define "prefer" as "to put before something else in one's liking or positive responsiveness,"

while select "as to pick out from among others on the basis of distinguishing qualities." However, there is the assumption that the insect can consider alternatives when responding to host cues.

Within the insect-host plant interaction, one of the difficulties in understanding host-plant location by insects, including Lepidoptera, is the classification of insects according to their diet breadth. The terms monophagy, oligophagy, and polyphagy are intangible to apply, since there is no set limit to categorize an insect. The term monophagous has been used to mean feeding on only one plant species, on plant species within one genus, or on plant species within one family (Dugdale, 1975; Slansky, 1976; Halloway and Hebert, 1979). Most classifications (Cates, 1980, 1981; Jones et al., 1987) categorize a monophagous species as one that utilizes one host plant species; while oligophagous species utilize several host species within a single plant family; and polyphagous species utilize hosts from different plant families. These categories are arbitrary, but provide a convenient set of terms, and are used as such here.

Theory of Host Location Mechanisms

Biotic and abiotic factors and the physiological state of the insect govern insect host location (Courtney and Kibota, 1990). Characteristics often recognized as factors in host-plant location are size, color, shape and chemistry

of plants. It has been demonstrated that chemical cues are important, and in some cases determinant, in locating a host (Thorsteinson, 1960; Chandler, 1969; Feeny et al., 1983; Renwick and Radke, 1987; Courtney and Kibota, 1990). Kogan (1977), however, considered that plant odors are of minor importance in host selection of some species, especially for r-strategists. Nevertheless, there has been a strong emphasis in research on the chemistry of insect-plant interactions. This focus follows the theory of Ehrlich and Raven (1964), who suggested that host chemistry has been the major force in evolution of diet of plant-feeding insects.

More recently, the importance of biotic factors when selecting and feeding on a host has been stressed (Smiley, 1978; Bernays and Graham, 1988), although the conviction remains that chemistry is the most important influence on plant selection behavior of insects (Ehrlich and Murphy, 1988; Schultz, 1988). Recent views propose an integration of plant chemistry with others mechanisms of host selection (Jermy, 1984; Barbosa, 1988; Courtney, 1988; Rausher, 1988; Thompson, 1988).

An early theory of plant selection was proposed by Brues (1920), who based host selection on "botanical instinct." The botanical instinct would compromise a mixture of identifying chemical and physical cues. Characteristics such as odor, taste, and visual aspects may be involved. Kennedy (1958) formulated the "dual discrimination theory," in which

both levels of nutrients and types of specific
allelochemicals determine host acceptance.

Additional levels for host discrimination were also
suggested by Thorsteinson (1960). Thorsteinson stressed the
importance of repellents and deterrents in host selection,
which had not been considered very important in earlier
theories.

One of the first models proposed for insect decision-
making was proposed by Fraenkel (1959, 1969), in which there
are "token stimuli" that elicit fixed behaviors toward a host
choice. In this model the insect works as a lock and the
stimuli as a key. However, this model has proved to be too
simplistic to explain insect host selection. Miller and
Strickler (1984) proposed the "rolling fulcrum" model based
on external and internal factors that may affect the insect's
decision. Excitatory and inhibitory inputs can be internal
or external. The internal factors determine whether the
external factors will cause acceptance or rejection. This
model is a mechanical analogue of the model proposed by
Dethier (1982), who focused on a chemical "Gestalt" and not
on single chemicals as determinants of acceptance. The model
has been used to explain behavior observed in several recent
studies (Wiklund, 1981; Singer, 1982, 1983; Singer at al.,
1988; Thompson, 1988).

Miller and Strickler's model (1984), sometimes referred
to as "black-box," does not consider alternative host
stimulation, processing of information, phylogeny, or

genetics controlling host selection. Nevertheless, it has been referred to as a very flexible model on which more complexities can be built, depending on the system (Courtney and Kibota, 1990). Rausher (1985) proposed both a mechanistic and an evolutionary model to explain the search for and selection of a host. The mechanistic model is based on insect learning (ontogeny) and the relative payoff sum rule (Harley, 1981) since one host or another will be chosen depending on past successes (nutritional value). On the other hand, the evolutionary model explains the dynamics of genes influencing search behavior and natural selection affecting such genes.

Courtney et al. (1989) proposed a hierarchy-threshold model, in which Tinbergen's causes affecting behavior are emphasized (Tinbergen, 1963). The causes integrated in this model are 1) proximate (eliciting and controlling factors), 2) ontogenetic (developmental changes, such as learning), 3) ultimate (survival value), and 4) phylogenetic (evolutionary history) factors. They suggest that these four factors should be integrated in host selection research. The principles of the "rolling fulcrum" model of Miller and Strickler (1984) are integrated into this model, which permits predictions of Tinbergen's levels mentioned previously. Such a rank-order acceptability of a host involves external stimuli and may include all of the senses. However, whether or not a host is accepted depends on internal factors, which set the motivational threshold for

acceptance (Courtney et al., 1989). The models mentioned use the term "host" in general; thus, they can be applied to oviposition or food selection.

Kennedy (1965) stressed that host selection is a catenary process involving a series of decisions to different stimuli that ultimately lead to the discovery and acceptance or rejection of the host. In addition, Rausher (1983) mentions the importance of habitat selection as prerequisite for host selection. Hanson (1983) states that host–plant selection by feeding insects is composed of three stages: (1) attraction to a potential food plant, (2) arrest or cessation of locomotion, and (3) stimulation or deterrence of feeding on that plant.

The olfactory detection and discrimination of plants from a distance have been objects of debate; however, there is little information available against or in favor of olfaction as a principal means of host location (Cardé, 1984; Kennedy, 1986; Finch, 1986). Kennedy (1986) suggested that distant orientation to host odor is due either to olfactory enhancement, or induction of visual orientation responses to single objects, or to odor-modulated optomotor anemotaxis. There is some evidence supporting these conclusions (Saxena et al., 1977; Visser (1986); Visser and Taanman, 1987). In addition, Singer (1986) states that it is difficult to determine if odor or visual characteristics are involved when an insect passes close by a host plant without responding.

There is evidence that host location and host acceptance are variable within and among populations (Papaj and Rausher, 1983). The variation may be due to genetic, experience (conditioning and learning), or physical factors (Tabashnik et al., 1981; Wasserman and Futuyma, 1981; Jaenike and Grimaldi, 1983; Smith and Cornell, 1979; Papaj and Rausher, 1983).

Host Finding and Host Selection in Lepidoptera

Brues (1920) suggested that the food plant of a lepidopterous larva is chosen by the adult female during oviposition and that larvae could not readily migrate to another plant even if they were willing to do so. Thus, the taste of the larva will match the maternal instinct of oviposition. The odor and taste would be part of the "botanical instinct" to recognize a host. In addition, the general form of the plant will also be important. Although this hypothesis that the gravid female makes the host plant choice is supported by various authors (Dethier, 1941a; Hanson, 1983; Fitt, 1991), there is evidence that Lepidoptera larvae are capable of recognizing a host plant by odor (Dethier, 1941b). In addition, host plant characteristics related to acceptability of a plant for feeding are not always correlated to those required for oviposition (Hsiao and Fraenkel, 1968; Pandey, 1977, Fitt, 1991).

Host selection by larvae has been studied primarily using feeding tests, in which contact cues are involved (Soo-

Hoo and Fraenkel, 1966; Schoonhoven, 1969; Pandey, 1977; Dethier and Kuch, 1971; Mitchell, 1977; Gerritis-Heybroek et al., 1978; Khan et al., 1987; Varela and Bernays, 1988). This tendency to focus on the last host-selection step as outlined by Miller and Strickler (1984) has been greatly influenced by the assumption that Lepidoptera larvae are not capable of locating host plants using odor cues, or that odor cues are unimportant in host selection (Dethier, 1937, 1988; Hanson, 1983; Fitt, 1991).

In lepidopterous larvae the antennae are responsible for sensing odors. It is believed that sixteen of the bipolar neurons in the antennae of caterpillars are olfactory receptors (Dethier, 1937, 1941b, Morita and Yamashita 1961; Schoonhoven and Dethier, 1966). The receptors are generalists, and overlap in their reaction. Thus, larvae in theory may be capable of discriminating more odors than would be encountered in a normal environment (Dethier and Schoonhoven, 1969).

Even though caterpillars possess a highly discriminatory olfactory system (Dethier, 1937; Dethier and Schoonhoven, 1969; Schoonhoven and Dethier, 1966), random movement and encounter of plants by chance are considered to be of major importance in larval host plant location (Rausher, 1981a; Dethier, 1988). In arctiid caterpillars the search behavior is primarily the result of physical features of the environment perturbing a central neuronal pattern of locomotion. Food plant stimuli have been thought to be

irrelevant to caterpillars prior to physical contact with the plant (Dethier, 1989).

On the other hand, there is indirect evidence indicating host plant location by caterpillars using cues from the resource. First instar *Helicoverpa zea* (Boddie) (Noctuidae), *Phthorimaea operculella* Zell. (Gelechiidae), *Chilo partellus* (Swinhoe) (Pyralidae), *Melitaea harrisii* Scud. (Nymphalidae), and second instar of *Pieris rapae* (Linn.) (Pieridae) are capable of finding the host (Dethier, 1959; Cain et al., 1985; Varela and Bernays, 1988; Terry et al., 1989; Saxena, 1990). However, it is not known which cues, if any, are used to locate the host plant. Furthermore, Hovanitz and Chang (1962) reported that *P. rapae* larvae are capable of detecting their preferred food plant from a distance of at least 120 mm and that wind movement was very important in locating the host, presumably through odor. The mechanisms responsible for host plant finding in *P. rapae* may well be chemical (chemotaxis) or odor conditioned upwind movement (chemoanemotaxis). In addition, first instar *Manduca sexta* (H.) (Sphingidae) (showed oriented movement as well as feeding responses to artificial diet, host, and some non-host plants and very low orientational and feeding responses to other non-host plants (Saxena and Schoonhoven, 1982).

Observations in the field also support the possibility of larval host plant finding. For example, the moth *Hemileuca oliviae* Cockerell (Saturniidae) lays eggs on non-host plants and the larvae actively search for the host plant

(Hansen et al., 1984). However, short range olfactory recognition of host plants may be sufficient for the larvae to find the host (Hansen et al., 1984).

Information available indicates that plant odors can be detected at distances from 5 mm to no more than 1 cm (Dethier, 1937, 1941a; Gupta and Thorsteinson, 1960; Saxena et al., 1977; Cain et al., 1985). However, silkworm larvae (*Bombyx mori* L., Bombycidae) are attracted to mulberry leaves placed within 3-4 cm. In addition, Watanabe (1958) reported that young larvae of *B. mori* tend to be attracted by ß-gamma-hexenol and to alpha-ß-hexanal. There is direct evidence showing larval attraction to a host in *Papilio demoleus* L. (Papilionidae) fifth instar larvae, which were attracted to constituents of host odors in a T-tube olfactometer (Saxena and Prabha, 1975). Also, neonate larvae of *Cydia pomonella* (L.) (Tortricidae), the codling moth, showed directional movement in still air toward apples when they were within 2 cm distance (Jackson, 1978).

Larval locomotion in relation to host location has also been studied in *P. rapae*, *Plusia californica* Speyer (Noctuidae) and *Plutella maculipennis* (Curt.) (Yponomeutidae) (Jones, 1977). Fourth and fifth instars were used for this study. *P. rapae* showed changes in searching patterns depending on hunger, whereas *P. californica* and *P. maculipennis* did not show substantial change in behavior as they starved. On the other hand, Chew (1974; in Chew, 1977) reported that first instar *Pieris* are not capable of locating

food plants more than a few centimeters away in the
laboratory and in the field. Thus, considering the
information available, it is evident that the knowledge of
host-finding behavior in Lepidoptera larvae is limited. Most
of the evidence of host finding behavior, including chemical
attraction, is indirect and the significance of any host
finding ability of larvae compared to that of the adult
female is essentially unknown.

On the contrary, most of the research on host-finding
mechanisms of insects has focused on the behavior of the
adult, specifically on oviposition and feeding by adults.
The oviposition behavior of butterflies was reviewed by Feeny
et al. (1983), with emphasis on Papilionidae. Oviposition
behavior in butterflies seems to be guided by visual cues,
both during habitat and host-plant search. Although a role
of olfaction during habitat and plant search has been
demonstrated for some butterflies (Brues, 1920; Mitchell,
1977, Saxena and Goyal, 1978), it has not been demonstrated
for other species (Feeny et al., 1983). Adult host location
in *Battus philenor* (Papilionidae) is based on visual cues at
a distance, and chemotactile and olfactory cues become
important when the female has landed on a plant (Rausher,
1978, 1981b; Papaj, 1986a,b).

Despite several reports of moth attraction to host odor,
there are still conflicting results. Ramaswamy (1988)
suggested that olfaction in *Heliothis virescens* (Guenée)
(Noctuidae) has no role in habitat or host finding. On the

other hand, Landolt (1989) and Tingle et al. (1989) demonstrated that *Trichoplusia ni* (Hübner) and *Heliothis subflexa* (Guenée), respectively, are attracted to host plant odors in a wind tunnel. In addition, the female of *H. subflexa* is able to respond to extracts of leaf washings from its host plant (Mitchell and Heath, 1987).

Some moths and butterflies lay eggs with high precision, in accordance with the suitability of the plants as food for the larvae (Merz, 1959 in Ehrlich and Raven, 1964; Leather, 1985, 1987; Firempong and Zalucky, 1990). On the other hand, for other species there are reports of a lack of correlation between oviposition and larval performance (Dethier, 1959; Saxena, 1978; Smiley, 1978; Wiklund, 1975; Rausher, 1979). Additionally, there are differences of host selection by adults within a species. For example, *Papilio machaon* L. (Papilionidae) females exhibited specialist and generalist oviposition behaviors in the same population. Generalists had a tendency to lay eggs on unsuitable food plants while specialist females were able to avoid oviposition on such plants (Wiklund, 1981). Similarly, *Pieris* oviposition choices are not always good hosts for larval development, even though in some cases larvae may feed or finish development on them (Chew, 1977). Furthermore, there are population differences in female searching behavior which determine the status of *P. rapae* as a pest or a minor nuisance (Jones and Ives, 1979). In *Pieris brassicae* (Linn.), the distribution of larvae on the host suggests that

larvae remain on the plant selected by the adult (Mitchell, 1977). The site of moth oviposition in *Chilo partellus* (Swinhoe) is not correlated with the plant suitability for neonate larvae (Ampofo and Nyangiri, 1986). It has been suggested that pre-alighting cues may be used to maximize oviposition rate and overall chance of larval survival, and post-alighting cues are mainly used to assess larval food quality (Rausher, 1983; Papaj and Rausher, 1983). Correlation between oviposition preference and larval performance has been studied in *Euphydryas editha* (Nymphalidae). This species showed that both preference and performance vary within a population with some individuals preferring and growing faster on one of two host plants (Singer et al., 1988). At the extreme of adult host selection are some species that do not select a host at all. Some Hesperiidae, Hepialidae, Arctiidae, and Megathymidae drop their eggs during flight, or deposit in the neighborhood of a food plant as in *Ornithoptera* (Papilionidae) and *Agathymus* (Megathymidae) (Schoonhoven, 1973; Stallings and Stallings, 1969; Straatman, 1969).

Thus, taking into account the reports and recent reviews on the topic, there is no consensus on whether the female makes suitable choices for its offspring. Nevertheless, Courtney and Kibota (1990) suggest that insects generally are poor in discerning suitable host plants for their offspring, a conclusion applicable in part to Lepidoptera (Thompson and

Pellmyr, 1991). Whether the mistakes of the adult females are real or intentional has yet to be determined.

Notwithstanding the efforts focused on host finding by adults, little attention has been directed to the role and capabilities of larvae to locate and select a host plant. The objective of this research was to study the potential for host finding and host selection of three species of noctuid moths with different diet breadth: the monophagous *Heliothis subflexa*, the moderately polyphagous species *Trichoplusia ni* (cabbage looper), and the polyphagous species *Spodoptera exigua* (beet armyworm).

Research Aims

Undoubtedly, the information available regarding host-plant insect interactions is increasingly growing. Nevertheless, the potential for host-finding by first instar larvae and the capabilities of displaced larvae to orient towards hosts have been neglected. This is due in part to early information indicating limited or no olfaction faculties of newly hatched larvae (Dethier, 1941a; Saxena et al., 1977; Hanson, 1983; Cain et al., 1985), as well as to the assumption that the adult exclusively chooses the host (Dethier, 1941a, Hanson, 1983, Fitt, 1991). Lately, there have been some advances in the study of larval host selection behavior, prompted by observation of inconsistencies in adult selection of oviposition sites. Nevertheless, studies have been limited to food selection at the plant surface and have

provided only indirect information on larval movement. A good portion of the knowledge on the subject has been provided by studies of butterflies, which cannot be applied necessarily to larval moth behavior. Furthermore, extensive information is needed to construct general models and interpretations of behavior and sensory capabilities of immatures.

The present study was undertaken to assess the survival, movement, and host plant attraction of *Heliothis subflexa*, *Trichoplusia ni* and *Spodoptera exigua*. The information gathered provides basic data for a better understanding of insect-plant relationships and the applicability of more effective control measures of insect pests.

The studies carried out were 1) hatching patterns, survival, and egg and larval weight; 2) horizontal and vertical movement; and 3) host plant attraction using a four-choice olfactometer.

CHAPTER 2
EGG AND LARVAL WEIGHT, HATCHING, AND SURVIVAL OF FIRST INSTAR
HELIOTHIS SUBFLEXA (GUENEE), *TRICHOPLUSIA NI* (HÜBNER), AND
SPODOPTERA EXIGUA (HÜBNER)

Introduction

Information on possible diel patterns of hatching and on
the lifespans of starved larvae was needed to plan further
experiments regarding the larval potential for host finding
and host location. In addition, information on egg and
larval weights was needed later to seek correlation with
information gathered during subsequent experiments. There is
limited published information on all aspects of the biology
of larvae of the three species studied. Although there is
more information available regarding the cabbage looper,
Trichoplusia ni (Shorey et al., 1962; Shorey, 1963), the
details necessary to design and perform the experiments
described herein were not available. Some of the limitations
were due to different experimental conditions (temperature,
humidity) or conflicting results with our preliminary
observations on the survival and behavior of newly hatched
larvae (Shorey et al., 1962).

T. ni has been reported to feed on over 160 species of
plants in 36 families (Sutherland and Greene, 1984).
However, Landolt and Lovvorn (1995, in preparation) have

reported that optimum larval performance is largely confined to plants of three families: Asteraceae, Cruciferae, and Umbelliferae, with hosts in the Asteraceae largely limited to the lettuce subfamily, Lactuceae.

T. ni oviposition takes place near dusk. Eggs are laid primarily on the lower surface of leaves, individually or in groups of two. Several eggs can be laid in proximity (Shorey et al., 1962; Shorey 1963, 1964). The number of eggs laid by a female in the laboratory varies from 50 to 1300 (McKinney, 1944; McEwen and Herbey, 1960; Shorey, 1963).

Duration of the egg stage in *T. ni* at 24°C is approximately 3 days (McEwen and Hervey, 1960). McKinney (1944) found that cabbage looper eggs take from 3 to 10 days to hatch depending on the temperature. A similar range of development to egg hatching was reported by Shorey et al. (1962). There are no reported studies of *T. ni* larval survival or eclosion patterns.

Published information on *H. subflexa* life history is generally limited, with virtually no reports on larval behavior or physiology. Brazzel et al. (1953) studied the *Heliothis* complex, including *H. subflexa,* larvae which occur exclusively on *Physalis* spp (Solanaceae). The most convenient larval character that separates *H. subflexa* from *Helicoverpa* (*Heliothis*) *zea* and *H. virescens* is the host specialization of *H. subflexa*. *H. subflexa* eggs are laid individually near or on the flowers. Newly hatched larvae burrow into the fruits and feed. One larva per fruit is

found in the field. Life cycle and oviposition behavior are similar to *Helicoverpa zea* and *H. virescens* (Brazzel et al., 1953; Hardwick, 1965).

There is little published information on larvae of the beet armyworm, *Spodoptera exigua*, a highly polyphagous moth that feeds on tropical and subtropical cultivated and wild plants (Taylor, 1931; Brown and Dewhurst, 1975). Among its hosts are sugar-beets, table beets, corn, cotton, peas, pepper, eucalyptus, celery, chrysanthemums, and tomato. Eggs are laid on the underside of leaves in batches of 50 to 150 and are covered with scales (Wilson, 1932). Wilson (1932) recorded 1171 eggs deposited by a single female. As in *T. ni* and *H. subflexa*, there is no published information for larval eclosion and survival of beet armyworm.

This chapter presents a study of preliminary basic information which will be utilized to investigate the movement capabilities and host plant attraction of *H. subflexa*, *T. ni*, and *S. exigua* larvae.

Materials and Methods

Eggs of cabbage looper (CL) and the beet armyworm (BAW) were obtained from the rearing facilities at the Insect Attractants, Basic Biology and Behavior Research Laboratories (IABBBRL, USDA), Gainesville, Florida. Adults of *T. ni* and *S. exigua* were maintained on a reversed 14:10 (L:D) light cycle. Eggs of *Heliothis subflexa* (HS) were obtained from a colony maintained for one year that was established from wild

larvae collected on *Physalis* spp. (Solanaceae). Experiments were performed at 24 ± 1°C and 60 ± 5% RH, and with a 14:10 (L:D) light cycle. Light during experiments was provided by overhead fluorescent lights (900 lux at the table top), unless otherwise specified.

Egg and Larval Weight

Fertilized eggs and 1-2 hour old larvae from the three species were weighed on an analytical balance (Mettler AE200). Due to the limits of readability of the balance (0.1 mg), 20 eggs were weighed initially and groups of 5 eggs were added and weighed until the number of eggs reached 50. This procedure was repeated three times for each species, providing 18 weighings of 5-egg batches per species. Egg batch egg weights were subjected to an ANOVA, and to the Fisher's Least Significant Difference (LSD) Multiple Comparison. To estimate individual egg weights, mean and SE egg batch weights were subsequently divided by 5.

Diel Hatching Patterns

Eggs from *T. ni* and *S. exigua* were monitored constantly with a video camera from acquisition of eggs from the insectary until all eggs hatched (up to 54 h and 24 h, respectively) to determine the time of egg hatch during both photophase and scotophase. One hundred eggs oviposited the day before were placed in a petri dish on damp black filter paper at 09h00 to facilitate the observation of hatching

eggs. The experiment was repeated three times for *T. ni* and
S. exigua. Experiments were performed at 24 ± 1°C and 60 ±
5% RH, and with a 14:10 (L:D) cycle. Light during the
photophase was provided by overhead fluorescent lights (900
lux at the table top), and during the scotophase by a lamp
with a red bulb (25W). Hatching in *H. subflexa* was not
studied due to a limited number of eggs obtained from the
colony.

Survival

The survival of starved larvae of the three species with
time was determined by placing 20 newly hatched larvae (1 h
old) individually in petri dishes (5 cm OD) (Falcon®) without
food or water, and recording the number of living larvae
every four hours until all larvae were dead. Prior to this
experiment, the empty egg shells were examined to determine
if larvae fed on them. Experiments were performed at 24 ±
1°C and 60 ± 5% RH, and with a 14:10 (L:D) cycle. The
experiment was repeated three times (n= 60). Number of
surviving larvae was analyzed with ANOVA and Fisher's LSD
Multiple Comparison.

Results

Egg and Larval Weight

The eggs of *H. subflexa* were the heaviest, followed by the
eggs of *T. ni* and *S. exigua*. The eggs of *H. subflexa*

averaged 77.8 ± 2.7 µg (SE). Eggs of *T. ni* were 65.6 ± 3.2 µg (SE), and eggs of *S. exigua* were 48.9 ± 2.9 µg (SE) (Figure 1). An ANOVA test and the Fisher's Least Significant Difference (LSD) Multiple Comparison showed that weights were statistically different (F= 24.33; d.f. 2, 51; P < 0.0001).

Newly eclosed larval weights for all three species showed differences similar to their egg weights (Figure 1). *H. subflexa* larvae weighed 57.8 ± 2.7 µg (SE), *T. ni* 48.9 ± 2.4 µg, and *S. exigua* 32.2 ± 2.4 µg. ANOVA and Fisher's Least Significant Difference (LSD) Multiple Comparison showed that larval weights were statistically different (F= 26.64; d.f. 2, 51; P= < 0.0001). There was a difference of approximately 17-20 µg between the egg and the newly hatched larva, which is likely attributable to the egg shell, and possibly to water loss.

Diel Hatching Patterns

Hatching of *T. ni* eggs was continuous during both the photophase and the scotophase (Figure 2). The number of *T. ni* eggs hatching showed a peak at 8-10 hours in the scotophase that was not statistically significant. The hatching of *S. exigua* also occurred throughout both photophase and scotophase (Figure 3). The highest numbers of *S. exigua* eggs hatched between 9 and 10 hours into the scotophase. There were no significant differences between egg hatching during the photophase and scotophase, for either *T. ni* or *S. exigua*.
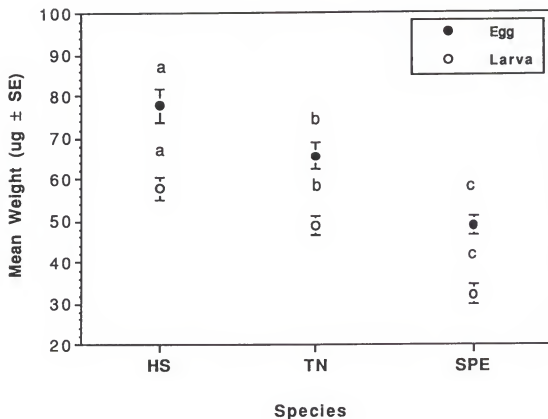
Figure 1. Egg and larval weight (Mean μg ± SE) of *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and *Spodoptera exigua* (SPE). N= 18 batches of eggs. Same letters indicate that values are not significantly different at 0.05 level of confidence (ANOVA and LSD Multiple Comparison test).

Figure 2. Percent of hatching (Mean ± SE) of *Trichoplusia ni* during photophase and scotophase (14:10). Difference between the two phases is not significantly different at 0.05 level of confidence ("t" test). N= 274.

26

Hours
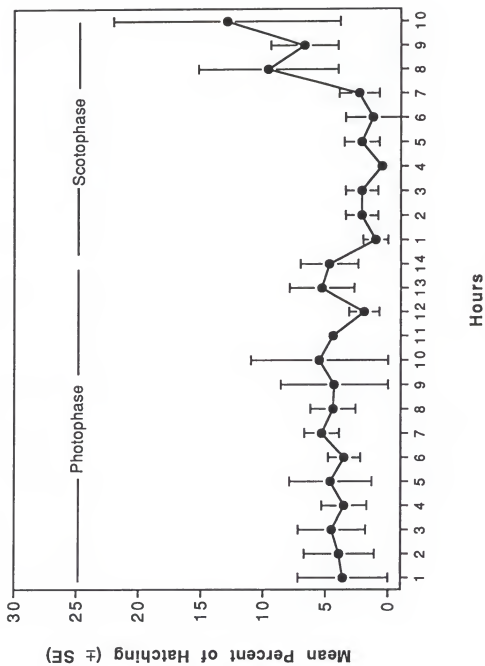
Figure 3. Percent of hatching (Mean ± SE) of *Spodoptera exigua* during photophase and scotophase (14:10). Difference between the two phases is not significantly different at 0.05 level of confidence ("t" test). N= 248.

28
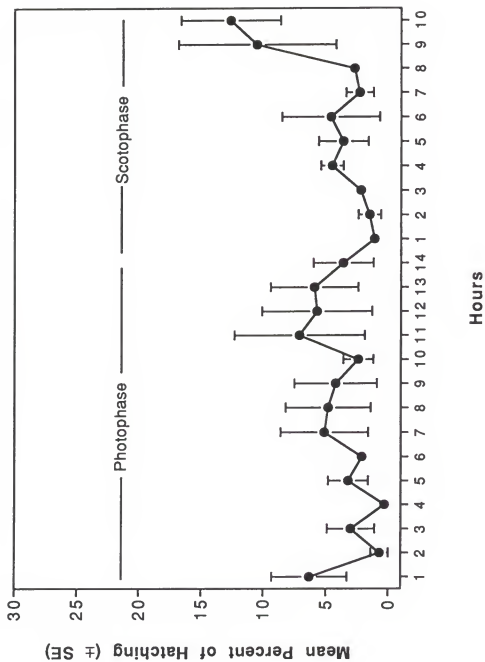
The information obtained during this experiment served to time egg hatching for the rest of the experiments, in which newly hatched larvae were required. Based on these data, additional tests with newly eclosed larvae of *T. ni* and *S. exigua* were conducted approximately 48 h and 24 h after obtaining eggs from the colonies, respectively. A total of 274 eggs of *T. ni* and 248 eggs of *S. exigua* hatched in the three repetitions per species.

Survival

Survival of starved newly hatched larvae of the three species was very similar the first 8 hours, but differed as time of starvation increased. The greatest difference was apparent at 28 hours after egg hatching, when a higher percentage of *H. subflexa* survived. This species was also the only one to survive up to 40 hours. *T. ni* and *S. exigua* larvae did not survive in this test longer than 36 hours (Figure 4). None of the species, *H. subflexa*, *T. ni* or *S. exigua*, fed on shell material or other larvae, even after approximately 10 hours of hatching. The survival data from the three species were not statistically different in an ANOVA test ($p = 0.05$).

Discussion

The lack of a clear diel pattern to hatching in *T. ni* and *S. exigua* indicates a lack of any circadian gate or control over larval eclosion from the egg. The timing of egg
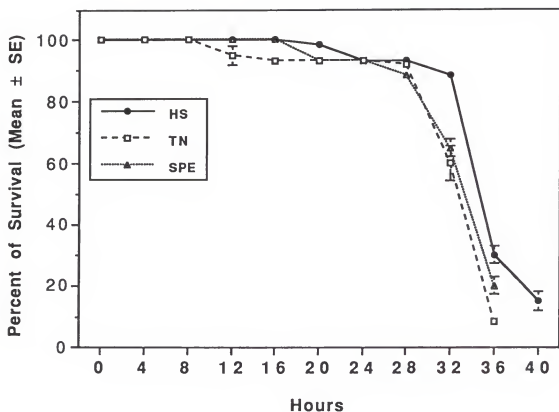
Figure 4. Percent survival of newly hatched larvae of
Heliothis subflexa (HS), *Trichoplusia ni* (TN), *and
Spodoptera exigua* (SPE) (n= 60). Differences among
species at each time period were not significantly
different at 0.05 level of confidence (ANOVA and LSD
Multiple Comparison test).

hatch may be a result of the timing of oviposition and egg development rate at a particular temperature.

The possible peak of hatching near dusk for *T. ni* and *S. exigua* may reflect earlier peaks in oviposition. It is knownthat *T. ni* moths lay most eggs shortly after sunset or during the first hours of the scotophase under laboratory conditions (Shorey et al., 1962). Any pattern of egg hatching may be the result of an earlier diel pattern to oviposition (Beck, 1980).

Egg hatching in both species apparently occurs with no relationship to differences in chances of larval survival under dark or light conditions. However, success in finding a host may depend on time of hatching. Larvae emerging in the scotophase may have a higher rate of survival than larvae emerging during the photophase, when higher temperatures and lower relative humidity increase the risk of desiccation.

Timing of subsequent experiments with newly hatched larvae was facilitated by knowing that the eggs would hatch approximately 48 hours (*T. ni*) and 24 hours (*S. exigua*) after obtaining them from the insectary.

The egg weights recorded for *T. ni* differed from that reported by Shorey (1963). Probably due to readability of the scale used, the egg weight reported by Shorey (1963) is slightly different (100 μg); 34 μg higher than the weight recorded during our experiments (64 μg).

None of the species consumed the egg shells after hatching. According to Shorey et al. (1962) *T. ni* eats the

remains of the egg before feeding on the plant. Wilson
(1932) described complete ingestion of the chorion by *S.*
exigua larvae immediately after hatching. In none of our
experiments did any of the species of larvae feed on the egg
shell, even several hours after hatching. There is no
evidence that the chorion, even when eaten, provides any
immediate survival benefit, at least in *T. ni* species (Shorey
et al., 1962). Other species such as *Spodoptera frugiperda*
also feed on the egg shell (Morrill and Greene, 1973). There
is the possibility that the chorion may give them
supplemental energy for locomotion while searching for a host
plant. Hinton (1981, and references therein) indicates that
the Lepidoptera egg shell contains amino acids, and that
chitin, in general, may be absent. On the other hand, there
is evidence that feeding in first instar *Agrotis orthogonia*
before starving can cause increased mortality due to
intestinal disturbance (Salt and Seamans, 1945).

The longevity or survivorship of starved, newly eclosed
larvae was similar for all three species tested. However, *H.*
subflexa on average survived slightly longer than *T. ni* and
S. exigua, a fact which may be correlated to the larger size
of the larva. There is some evidence showing that survival
time and size of the lepidopteran egg and larva is directly
correlated; large larvae live longer than small ones
(Richards, 1959; Taber and Roberts, 1963; Jones, 1977; Derr
et al., 1981; Slansky and Scriber, 1985; Stockhoff, 1991).
For example, starved first instar larvae of *Lymantria dispar*

lived 4.3-4.8 days. First instar larvae of this species weighs 0.47-0.64 mg (Capinera and Barbosa, 1976), which is seven to ten times the weight of first instar *H. subflexa, T. ni and S. exigua*, respectively. Large larvae of *Heliothis armigera* are less eager to eat than the smaller larvae, which suggests that larger larvae may resist starvation better (Barber, 1941). Nevertheless, larval body weight does not affect survival of newly hatched satyrid species (Karlsson and Wiklund, 1984, 1985).

Thus, egg and larval weight differences among the three species studied do not explain the survival time observed. Consequently, survival of starved larvae may or may not be correlated to the quantity of their energy reserves.

The times that starved larvae of the three species survived were similar to the results reported for starved first instar larvae of *Spodoptera frugiperda* and *Chilo partellus* (Swinhoe) under similar conditions (Morrill and Greene, 1973; Berger, 1992). However, Shorey et al. (1962) reported that newly hatched larvae of *T. ni* survived for 2.3 to 4 days in temperatures ranging from 14-32°C, while in our experiments larvae only lived up to 36 hours at 25 ± 1°C.

Mortality of larvae of the three species (*H. subflexa, T. ni*, and *S. exigua*) in this test was likely due to starvation and desiccation. The fact that the larvae have a high surface area to volume ratio increases water evaporation and as a consequence increases mortality. For instance, death for the codling moth first instar, *Laspeyresia*

pomonella (L.), was attributed to starvation and dehydration (Jackson, 1980). Yet, resistance to desiccation is not always strictly related to size, since specific physiological and behavioral adaptations to dry conditions can affect this relationship (Karlsson and Wiklund, 1985).

The polyphagous *T. ni* and *S. exigua* exhibited a slightly shorter mean starvation survival than the monophagous *H. subflexa*, although not statistically different. However, we expected significant differences in survival rate among the three species due to differences in host specialization. Survival of *H. subflexa* was expected to be short since the egg is placed on the fruit or leaves of the host plant by the moth. Consequently, the larvae should not need large reserves of energy or a lot of time for searching for food. Reavey (1993) stated that eggs laid on the plant part that is eaten tend to be smaller than those laid away from it. However, the longer lifespan of *H. subflexa* may be an ancestral character, since monophagy is considered to have evolved secondarily to polyphagy (Futuyma, 1976; Rhoades and Cates, 1976).

The three species of noctuids studied do not follow a pattern related to host breadth reported for other Lepidoptera species. Reavey (1993) found that within Lepidoptera families, eggs (and therefore newly hatched larvae) of generalist feeders are larger than those of specialists. Furthermore, Reavey (1993) reported that there is no correlation between size of newly hatched larvae and

survival time or between larval survival and feeding specificity. Although the difference of survival time among the species included in the present study is not statistically significant, it may be biologically significant at the time of searching for a host. Longer survival, even a few hours, may be critical for a caterpillar to successfully find a host.

CHAPTER 3

VERTICAL AND HORIZONTAL MOVEMENT OF FIRST INSTAR
HELIOTHIS SUBFLEXA (GUENEE), *TRICHOPLUSIA NI* (HÜBNER), AND
SPODOPTERA EXIGUA (HÜBNER) IN THE LABORATORY

Introduction

Quantitative and qualitative analyses of movement of first instar larvae of Lepidoptera in the laboratory and in the field are scarce. Most information regarding larval movement is indirect, and in some cases contradictory with other studies. For example, Alghali and Saxena (1988) reported that first instar *Chilo partellus* Swinhoe did not perform interplant movement before initial feeding in sorghum cultivars. However, other authors (Chapman et al., 1983; Ampofo and Nyangiri, 1986; Saxena, 1990) reported caterpillar movement to adjacent plants. Some species of butterflies also show interplant movement, but the strategies of movement used to reach a host are not known (Dethier, 1959; Cain et al., 1985).

Search mechanisms in adult insects, on the other hand, have prompted a great volume of research. Behavioral studies have often involved predators seeking prey (Bond, 1980; Carter and Dixon, 1982), parasitoids searching for hosts (e.g. Waage, 1978) or response to volatile sex pheromones (Bell and Tobin, 1981).

Studies of host plant selection indicating host finding by lepidopterous larvae are scarce. In part, this may be due to the conjecture that host selection by caterpillars, in general, is absent or limited and that adults are responsible for host selection. However, there is abundant evidence to the contrary. There are some Hesperiidae, Hepialidae, and Arctiidae that drop the eggs during flight. Other species of Lepidoptera deposit the eggs in the neighborhood of a food plant (Stallings and Stallings, 1969; Straatman, 1969; Schoonhoven, 1973). In addition, there is evidence of mistaken oviposition on non-host plants (Dethier, 1959; Straatman and Nieuwenhuis, 1961). Also, even if the plant is an appropriate host, the larvae may become detached or the plant completely devoured, necessitating movement to another plant.

Host finding ability in the three species studied may be related to their diet breadth and the specificity of oviposition cues for adult females. *H. subflexa* host range is constrained to *Physalis* spp., both by the host selection behavior of the adult (Mitchell and Heath, 1987; Tingle et al., 1989) and by physiological adaptations of the larvae to the host plant (Brazzel et al., 1953; Laster et al., 1982; Fitt, 1991). In the field, larvae are recorded only on *Physalis* spp. (Brazzel et al., 1953; Laster et al., 1982). *Trichoplusia ni* and *Spodoptera exigua* have been recorded on a large number of taxonomically diverse plants (Wilson, 1932; Sutherland and Green 1984), although this does not mean that

all are suitable hosts (Landolt and Lovvorn, 1995, in preparation). Female *T. ni* moths were attracted to, and oviposited on, any of a number of different host plants (Landolt, 1989). Consequently, based on ecological and adult behavioral constraints, larvae of polyphagous species may need behavioral mechanisms to orient to and to reach (by movement) a host plant.

Yano (1978) proposed that larval searching behavior can be regarded as a process of functional response with two basic components, speed of movement and relative change of direction of movement. We can summarize the problem as follows. The movement parameters of lepidopterous larvae that may be involved in search for a host are: 1) locomotory rate, 2) turning rate, 3) turn dimensions, 4) turn direction or bias, 5) stop-run pattern, and 6) the release of specific motor functions such as casting or turning on axis (Bell et al., 1985)

Jander (1975) defines movement patterns such as ranging and local searching as two categories of searching orientation. Ranging is also referred to as extensive searching (Bond, 1980), which describes orientation between resources or resource patches and is characterized by a relative straight course. Local searching, also referred to as intensive searching (Bond, 1980), or area-restricted searching (Hassell and May 1974; Curio, 1976), describes orientation within a resource patch, and is characterized by looping, intersecting, convoluted orientation patterns.

Except for the perception of the initial cue, which is based on external sensory information, local searching appears to be driven entirely by internal information that is genetically stored or learned (Bell and Tobin, 1982).

The aim of the present research is to study the movement capabilities and movement patterns, vertical and horizontal, of starved first instar larvae of *Heliothis subflexa*, *Trichoplusia ni* and *Spodoptera exigua*, which exhibit monophagy, moderate polyphagy and polyphagy, respectively. This would allow us to better understand the potential of host plant selection by newly hatched larvae.

Materials and Methods

Vertical Movement

Heliothis subflexa, *Trichoplusia ni* and *Spodoptera exigua* newly hatched larvae, 1-2 hours old, were placed individually at the base of vertical glass tubes (120 cm, 3 mm OD). Larvae were handled carefully with a fine paint brush. Using a grid (1 cm) on a white background, the vertical position of each larva on a tube was noted and recorded at 3-minute intervals. Tubes were turned over when larvae reached the top. Fallen larvae were picked up with the brush and returned to the last recorded position. Larval positions on the tubes were recorded for one hour every six hours until the larvae did not move or died. During each test, 20 larvae were evaluated simultaneously, all of one

species. This test was repeated three times for each species, with a total of 60 larvae evaluated. Larvae were kept at 24 ± 1°C and 60 ± 5% RH, under 14:10 light-dark (L:D) cycle. Light during photophase and during the experiment was provided by overhead fluorescent bulbs (900 lux at the table top).

Larvae were kept without food in plastic petri dishes (5 cm OD) (Falcon®) between recordings. The results are presented as the mean of the total distance traveled by each larva per hour.

Horizontal Movement

Horizontal movement of newly hatched larvae (1-2 hours old) was measured on a white paper (120 x 100 cm) which served as an arena. The individual pathways of ten larvae per species, one larva at a time, were drawn with a pencil. The movement was recorded for one hour every six hours until the larva did not move within an hour or died. Larvae that left the arena were returned to the center of the arena using a fine paint brush. The results are presented as the mean of the total distance traveled by each larva. Larvae were kept at 24 ± 1°C and 60 ± 5% RH, and with a 14:10 (L:D) cycle. Light during the experiment and photophase was provided by overhead fluorescent bulbs (900 lux at the table top). Larvae were kept without food in plastic petri dishes (5 cm OD) (Falcon®) between recordings.

The length and angular change of the path traced was measured for each larva. The length was measured with a map-measurer (cm), and the angular change was measured by drawing tangents to the trace and measuring angles with a protractor. Angular change was also quantified as total left turns and total right turns. A turn was considered complete when the larva changed its direction either to the right or to the left. The rate of turning (deg/cm) is reported for each species. The rate of turning was calculated by dividing the total angular change by the distance traveled in one hour. Analysis (paired "t" test) of right and left rate of turning were performed for the three species. ANOVA test and LSD Multiple Comparison were performed to compare mean rate of turning among species.

All the statistical analyses were done with JMP-IN™ software (SAS Institute Inc.) on a Macintosh LC.

<div align="center">Results</div>

Vertical Movement

H. subflexa traveled the longest distance of the three species (173.9 ± 5.2 (SE) cm/h). *T. ni*, on average, moved intermediate distances (152.2 ± 6.1 (SE) cm/h) and larvae of *S. exigua* moved the least (67.7 ± 4 (SE) cm/h) (Figure 5). Movement of larvae in some instances was downwards. An ANOVA test and the Fisher's Least Significant Difference (LSD) Multiple Comparison of the overall distance traveled showed

significant differences between distance traveled of *H. subflexa* and *S. exigua* (P <0.01, 2 d.f.). *T. ni* movement was also different from *S. exigua* (P <0.05, 2 d.f.), but not from *H. subflexa*.

Changes in the rate of movement over time were somewhat similar for the three species; these changes were, of course, correlated with the age of the larvae. Generally, motility decreased as the larvae aged without food. *T. ni* and *S. exigua* exhibited a higher motility at 6 hours of age, 205.9 ± 7.8 (SE) cm/h and 101.2 ± 7.2 (SE) cm/h, respectively, compared to 0 and 12 hours of age. After this peak, the distance traveled decreased as the larvae aged. On the other hand, *H. subflexa* traveled the most at 0 hours, 237.8 ± 9.3 (SE) cm/h, after which the motility generally progressively decreased (Figure 6). The decreased movement of *S. exigua* after 6 hours was less pronounced than those of the other two species, suggesting a more sustained rate of movement as larvae aged.

As in the previous experiment which determined the longevity of starved larvae, *H. subflexa* in this experiment survived longer on average than *T. ni* and *S. exigua*. Mortality occurred principally after 24 hours, but some larvae (13.3%) were still moving up to 36 hours after eclosion. On the other hand, substantial larval mortality of *T. ni* and *S. exigua* was already apparent at 6 hours, and increased as the larvae aged. *S. exigua* larvae showed lower
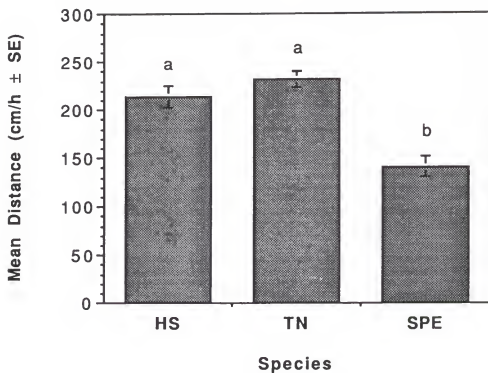
Figure 5. Total mean vertical movement (cm/h) of first instar *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and *Spodoptera exigua* (SPE) (n= 60). Same letters indicate that values are not significantly different at 0.05 level of confidence (ANOVA, LSD multiple comparison test).
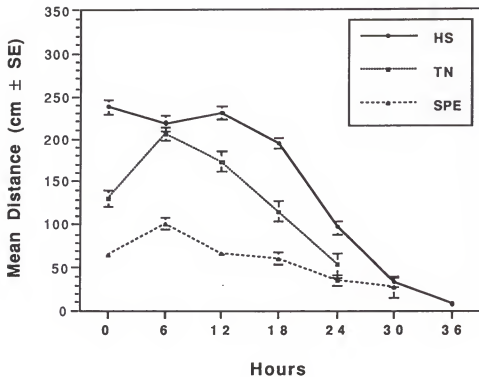
Figure 6. Vertical movement (cm) by hour of first instar *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and *Spodoptera exigua* (SPE) (n= 60).

mortality than *T. ni*, and 22.2 % lived up to 30 hours (Figure 7). Larvae of *H. subflexa* and *T. ni* when disturbed would release a thread of silk to lower themselves. This behavior was not observed in *S. exigua* larvae.

Horizontal Movement

H. subflexa larvae (n= 10) traveled a mean of 214.1 ± 11.3 (SE) cm/h, while larvae of *T. ni* (n= 10) and *S. exigua* (n= 10) traveled 232.3 ± 8.4 (SE) cm/h and 141.1 ± 10 (SE) cm/h, respectively (Figure 8). Larvae of all three species generally moved continuously during the experiment. Short stops were observed when larvae were close to death. The three species showed similar patterns in movement rates over the duration of the experiment. The distance traveled by all species showed a peak at 6 hours, decreasing as the larvae aged. *T. ni* and *H. subflexa* traveled similar distances at 6 hours: 301.4 ± 13.2 (SE) cm/h and 296.3 ± 20.2 (SE) cm/h, respectively. *S. exigua*, in contrast, traveled a mean of 173.9 ± 14.3 (SE) cm/h (Figure 9) at 6 hours into the experiment. Also, the movement pattern of *S. exigua*, although inferior to the other two species, showed a less pronounced decline after 6 hours.

A comparison of the overall rates of turning of the three species suggests that *H. subflexa* (44.3 ± 3.7 (SE) deg/cm·h) moved in a straighter fashion than *T. ni* (58.0 ± 8.9 (SE) deg/cm·h) and *S. exigua* 60.8 ± 9.5 (SE) deg/cm·h) (Figure 10).

Figure 7. Percent survival of newly hatched larvae of
Heliothis subflexa (HS), *Trichoplusia ni* (TN), and
Spodoptera exigua (SPE) during vertical movement
(n= 60).

Figure 8. Total mean horizontal movement (cm/h) of first instar *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and *Spodoptera exigua* (SPE). N= 10 for each species. Same letters indicate that values are not significantly different at 0.05 level of confidence (ANOVA, LSD multiple comparison test).
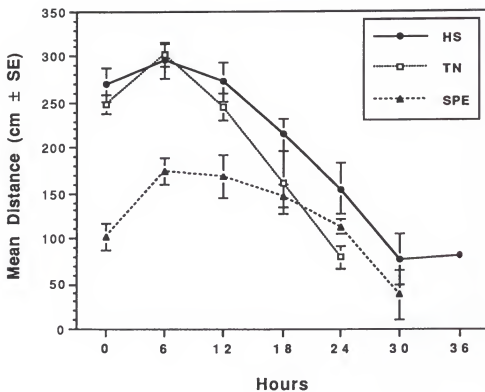
Figure 9. Horizontal movement (cm) by hour of first instar *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), *and Spodoptera exigua* (SPE). N= 10 for each species.
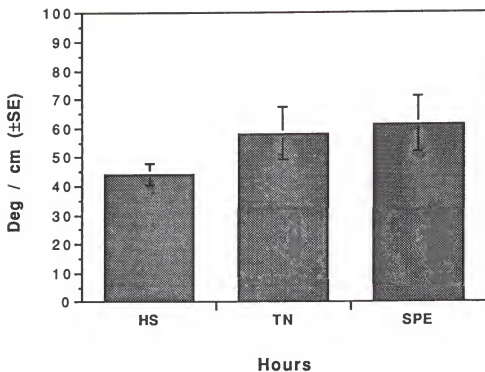
Figure 10. Overall rate of turning (deg/cm) of first instar *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and *Spodoptera exigua* (SPE). N= 10 for each species. Values were not significantly different at 0.05 level of confidence (ANOVA, LSD Multiple Comparison test).

However, the differences were not statistically significant.
The hourly pattern of the rate of turning for *H. subflexa* and
S. exigua were similar. On the other hand, *T. ni* showed a
progressive increase as the larvae aged (Figure 11).

The results of right and left (R:L) turning suggested a
bias to the right for *H. subflexa* (29.1 ± 4.6 (SE): 15.2 ±
3.4 (SE) deg/cm·h), and *S. exigua* (41.2 ± 9.8 (SE): 19.5 ±
6.7 (SE) deg/cm·h), and to the left for *T. ni* (19.7 ± 8.7
(SE): 38.3 ± 9.7 (SE) deg/cm·h) (Figure 12). The difference
between right and left rate of turning was not statistically
different in any of the species. The tendency of turning,
either to the right or to the left, was maintained throughout
the experiment in all three species (Figure 13-15).
The survivorship of the three species was similar. However,
H. subflexa showed lower mortality than the other two species
during the experiment, and larvae were still alive at 36
hours. Mortality of *T. ni* and *S. exigua* began early in the
experiment, and larvae only lived up to 24 and 30 hours,
respectively (Figure 16).

Discussion

There has been little consideration given in prior research
of host finding to possible roles of movement of first instar
larvae of herbivorous insects. In part, this lack of
information on larvae is due to the assumption that adults
select the host plant (Merz, 1959; in Ehrlich and Raven,
1964; Leather, 1985, 1987; Firempong and Zalucky, 1990),
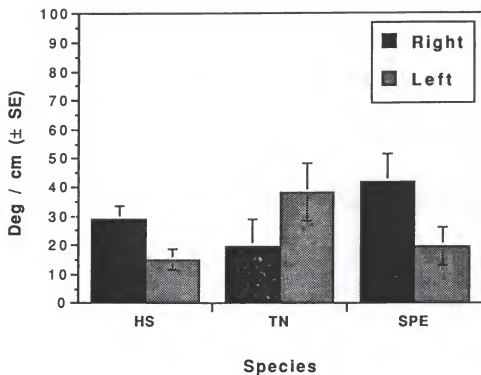
Figure 11. Overall right and left rate of turning (deg/cm) of first instar of *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and *Spodoptera exigua* (SPE). N= 10 for each species.
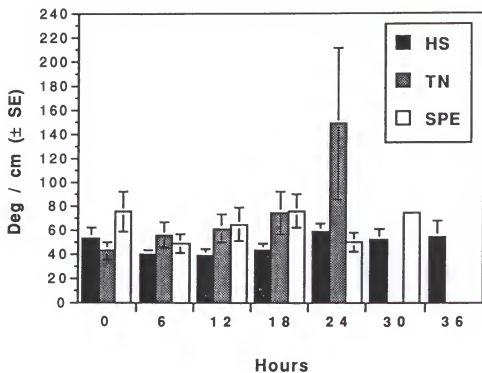
Figure 12. Hourly total (right + left) rate of turning (deg/cm) of first instar *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and *Spodoptera exigua* (SPE). N= 10 for each species.

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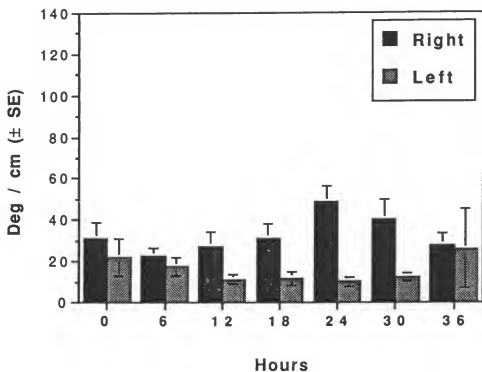
Figure 13. Hourly right and left rate of turning
(deg/cm) of first instar *Heliothis subflexa* (n= 10).
Values were not significantly different at 0.05 level
of confidence ("t" test).
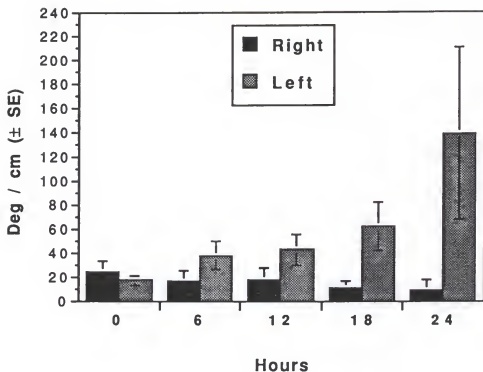
Figure 14. Hourly right and left rate of turning (deg/cm) of first instar *Trichoplusia ni* (n= 10). Values were not significantly different at 0.05 level of confidence ("t" test).
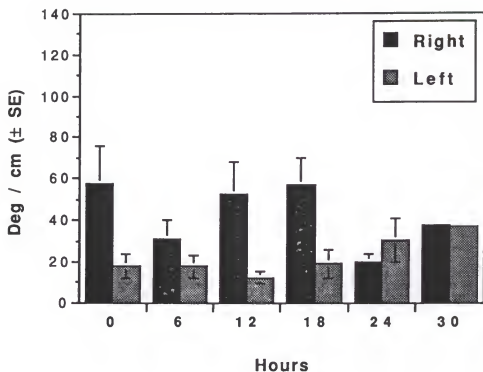
Figure 15. Hourly right and left rate of turning
(deg/cm) of first instar *Spodoptera exigua* (n= 10).
At 30 hours there was only one larva. Values were
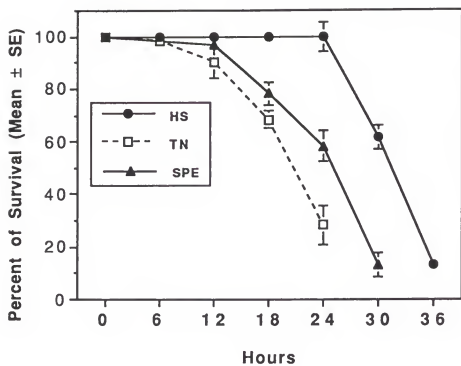not significantly different at 0.05 level of
confidence ("t" test).
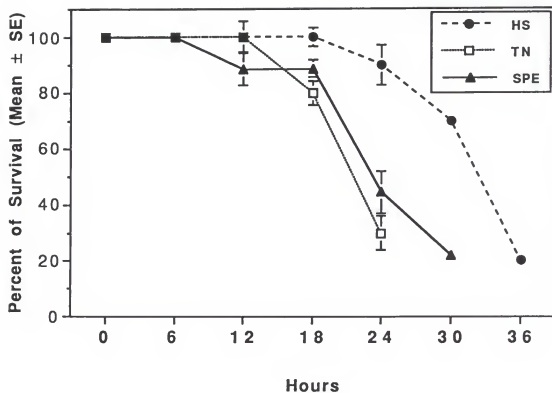
Figure 16. Percent survival of newly hatched larvae
of *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and
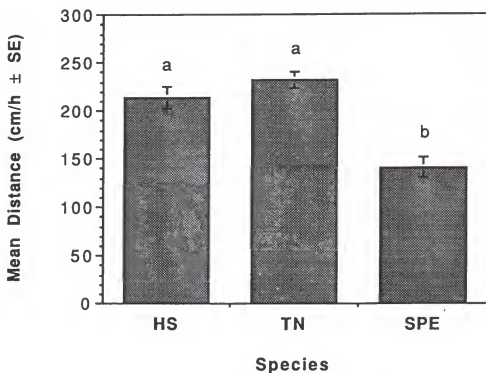*Spodoptera exigua* (SPE) during horizontal movement.
N= 10 for each species.

although oviposition on nonhosts has been reported (Dethier, 1959; Rausher, 1979; Chew, 1975; Saxena, 1978; Smiley, 1978).

According to the results obtained during the present investigation, newly hatched larvae of *H. subflexa*, *T. ni*, and *S. exigua* can travel considerable distances under laboratory conditions. Interpolating the data of the hourly quantification of movement, first instar *H. subflexa*, *T. ni* and *S. exigua* could travel up to 7,020 cm, 5,212 cm, and 4,079 cm, respectively, between egg hatch and death from starvation or desiccation. Although it was not quantified, it was obvious that larvae of all three species moved continuously during observations, except when they were close to death during the last hour of observation. Reports of movement in other moth larvae did not include this aspect (Jones, 1977; Dethier, 1989). Available data on time spent moving by apterous *Drosophila melanogaster* and *Phormia regina* show high variation in time spent moving during starvation (Green, 1964; Bell et al., 1985).

The ability of *H. subflexa*, *T. ni*, and *S. exigua* to move distances is greater than formerly thought. The potential for host finding by first instar larvae is significant. It is known that larvae of some Lepidoptera such as *Lymantria dispar* (L.) and *Spodoptera exempta* (Capinera and Barbosa, 1976; Ma, 1976), may disperse when not on a good host. The potential benefits from dispersal will increase as the suitability of the occupied host plant decreases (Capinera and Barbosa, 1976) . However, there will be trade-offs with

this behavior since the wandering larvae may never find a suitable host. Thus, natural selection would favor searching mechanisms that maximize the benefits of searching and would reduce the risks while searching (Bell, 1990). Differences in dispersal abilities among individuals of a brood have been shown to encourage colonization of new sites while allowing for the exploitation of suitable but uncrowded habitats (Wellington, 1957, 1960, 1964, 1965). Larvae of *Melitaea harrisii* could not reach plants at distances more than 120 cm, and mortality during migration was very high (Dethier, 1959). Nevertheless, dispersal in the face of optimal conditions has also been suggested by some authors as a method of population regulation (Lidicker, 1962; Southwood, 1962).

The differences in capability of movement observed among *H. subflexa*, *T. ni*, and *S. exigua* apparently are related to differences in larval size and not to oviposition specificity of the adult or diet breadth of the larvae. Horizontal and vertical movement are correlated with size of the larvae (Figure 17). *H. subflexa* (0.06 mg) moved a mean of 219.6 cm/h, *T. ni* (0.05 mg) moved a mean of 186.7 cm/h, and *S. exigua* (0.03 mg) moved a mean of 114 cm/h. Based on these results, it is evident that larval motility capability is not related to oviposition specificity by the female. *H. subflexa* would be expected to travel the least and *S. exigua* the most to correspond to possible larval needs to move to suitable feeding sites. On the other hand, if motility is related to

larval diet breadth, *S. exigua* would be expected to travel the least to locate a suitable host and *H. subflexa* to travel the most on average before encountering a host.

With respect to a correlation between diet breadth and larval search ability, Reavey (1993) did not find evidence that larvae of species that oviposit away from larval food are more active or are active longer compared to larvae of species that oviposit directly on larval food. Moreover, Reavey (1993) reported that there was no correlation between first instar larval size and survival time, or between larval size and speed of movement. In Reavey's studies (1993), the smallest caterpillar studied, *Pterophorus pentadactyla* (Pterophoridae) (0.022 mg) moved 34 cm h^{-1}, and the largest was the obscure wainscot *Mythimna obsoleta* (Noctuidae) (0.17 mg), which moved at 268 cm h^{-1}.

The gradual decrease in motility of the three species, *H. subflexa*, *T. ni* and *S. exigua*, throughout the experiment is similar to that observed in other insects. A decline in the rate of movement may be explained by an increase of energy expenditure or decrease in energy availability. Bell et al. (1985) and Green (1964) reported that in starved Diptera there is a peak of energy expenditure as time of starvation increases, and then the energy expenditure declines. Starved larvae of *H. subflexa*, *T. ni*, and *S. exigua* may have traveled less distance and increased the rate of turning after 12 hours posteclosion in order to conserve energy and postpone death from starvation.
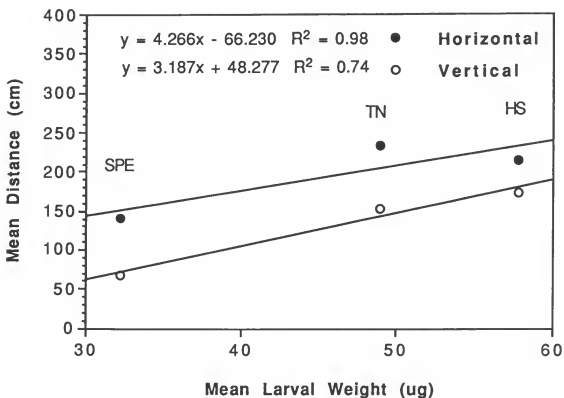
Figure 17. Relationship between larval weight and horizontal and vertical distance traveled (cm). *Heliothis subflexa* (HS), *Trichoplusia ni* (TN), and *Spodoptera exigua* (SPE).

The increase in rate of turning by larvae with time resulted in the limiting of their paths to a small area for all three species studied. Apparently, the metabolism of energy reserves and the behavior of starved insects are similar in these moths. The decreased distance traveled with an increased rate of turning has been suggested to be adaptive for at least two reasons. First, in the absence of information regarding a host plant, ranging (straight line travel) should diminish as the period of starvation increases. This is consistent with optimal foraging theory (e.g., Pyke et al., 1977) in that organisms should spend more time within patches as the cost of moving to another patch increases (Zimmerman, 1981). Second, additional host plants may be detected through odors. Thus, the adaptive value of this searching behavior may well also be applied to first instar *T. ni*, which exhibited an increased rate of turning and would possess a refined sense of olfaction as reported in other Lepidoptera species (Dethier, 1937; Dethier and Schoonhoven, 1969; Schoonhoven and Dethier, 1966).

The general pattern of a gradual increase in rate of turning exhibited by newly hatched starved larvae of *T. ni* fits that observed in other insect species. *Melitaea harrisii* (Nymphalidae) (Dethier, 1959) and apterous *Drosophila melanogaster* (Bell et al., 1985) increased the rate of turning as the starvation time increased. On the other hand, a pattern of increased tortuosity (deg/cm during forward movement) with age was not noticeable in *H. subflexa*

and *S. exigua*. Such behavior has been reported for other insects. Starvation did not influence temporal patterns of locomotion of arctiid caterpillars on a smooth surface or in clumped *Plantago* sp. in the field (Dethier, 1989). In addition, starvation in larvae of *Plusia californica* (Noctuidae) and *Plutella maculipennis* (Yponomeutidae) (Jones, 1977), lacewing larvae (Bond, 1980), and coccinelid larvae (Carter and Dixon, 1982) did not have a significant effect on rate of turning. On the contrary, hungry *Pieris rapae* fifth instar larvae moved faster and in a straighter path, and when replete with food moved slowly and turned often (Jones, 1977). It is necessary to point out that there are differences of searching behavior not only between species, but also within the same species (Jones and Ives, 1979; Bauer and Sokolowski, 1984). Variation in behavior among individuals of the same species was not addressed in the present project, but may occur with the three species of moths studied.

The patterns of movement in the three species illustrate the diversity of responses, ranging (extensive searching) and local searching (intensive searching), to internal factors (hunger) when searching for a host. According to Jones (1977), for phytophagous species that feed on plants that are distributed in small clumps (*Pieris* and *Plutella*), it would be advantageous for an insect to search areas thoroughly (local searching). On the other hand, a polyphagous species with scattered resources would be more successful in locating

a host if it searched large areas less thoroughly (extensive searching) (Kitching, 1971; Jander, 1975). The present results appear to contradict this hypothesis: *H. subflexa* (monophagous) exhibited a straighter pattern of movement than *T. ni* and *S. exigua* (polyphagous). On the other hand, this character of *H. subflexa* may be ancestral, since monophagy is considered to have evolved from polyphagy (Schoonhoven, 1973; Futuyma, 1976; Rhoades and Cates, 1976).

Jander (1975) has noted that the most efficient strategy when no information about spatial location is available, and when the resources are patchy, is straight-line progression (transecting). On the other hand, straight line movement would eventually direct an organism out of its habitat. In that case, *H. subflexa* would be more efficient than *T. ni* and *S exigua*, since the results suggest that larvae of *H. subflexa* travel in a straighter pattern than *T. ni* and *S. exigua*.

An arbitrary and subjective division of the turning behavior observed in the three species may be suggested. The gradual increase in the rate of turning observed in *T. ni* larvae may be interpreted as follows: at the beginning, the insect "ranges" in a relatively straight line and later switches to "searching." The latter behavior in *Blattella germanica* is exhibited when no stimuli are encountered (Schal et al., 1983). On the other hand, the behavior exhibited by larvae of *H. subflexa* and *S. exigua* may be described as "searching," "ranging," and "searching" again. These

behaviors are considered to be genetically stored but released by external sensory information (Jander, 1975; Bell and Tobin, 1982).

The tendency to turn more, either right or left, by the three species showed a defined pattern in *T. ni* larvae. The left-handed tendency exhibited by *T. ni* larvae when turning increased with age. On the other hand, the right-handed tendency exhibited in the turning by *H. subflexa* and *S. exigua* did not show a correlation with age of the larvae. This behavior has been reported in apterous *D. melanogaster*, which turns approximately equally to the left or right through the first 12 hours of starvation. However, after further starvation, *D. melanogaster* flies exhibited a significant turning bias (Bell et al., 1985).

The biological significance of the right- or left-handed bias in turning of *H. subflexa*, *T. ni*, and *S. exigua* larvae, and any role in mechanisms of host-searching behavior, remain to be investigated. The pattern observed in the three species of larvae may be genetically stored, since *T. ni* larvae exhibited different behavior from the other two species (*H. subflexa* and *S. exigua*) under the same environmental conditions.

Apparently, directional photic stimulation is a major modifier of locomotion (Wiener and Norris, 1982). It has also been suggested that polarized light could be employed as a navigational cue (Wellington et al., 1951; Dethier, 1989). Since caterpillars do not use light-compass reactions for

navigation to or from a fixed point, as in homing, the
results suggest that the external source of directional
stimulation might serve as a means of maintaining linearity
(Dethier, 1989). Although we could not see any indication of
orientation towards any particular visual feature during our
experiments, crude form perception has been demonstrated in
caterpillars (Dethier, 1943; Doane and Leonard, 1975). It
has been suggested that form perception could also serve to
orient caterpillars and keep them on a straight course, but
the points of reference would have to change as each landmark
passed. However, its effective range is so short, 30 cm,
that it would be ineffective in maintaining linear locomotion
for large distance (Ichikawa and Tateda, 1982). The role of
the movement patterns of caterpillars in host plant finding
has been characterized as fortuitous. According to Dethier
(1988, 1989), results of locomotion, sensory physiology, and
foraging behavior of arctiid larvae suggest that what appears
to be food-related search behavior is in fact the result of
insect response to physical features of the environment
(characteristics of the substrate, obstacles, light). These
physical features will perturb a central neuronal programmed
pattern of locomotion.

In summary, the movement pattern observed in insects is
the response to a combination of biological characteristics
of the insect and to external (environmental) and internal
factors (proprioceptive and genetic information) (Bell,
1990).

The ability of caterpillars to search has trade-offs, since there is high risk of mortality. Gadgil (1971) and others have stated that there must be overriding advantages favoring the tendency to disperse, due to the high risks of mortality of dispersants. Movement is risky, and searching by first instar larvae may not be profitable because of the many predators and their small size (Dempster, 1967; Scriber and Slansky, 1981). However, traveling and selecting a good host rather than feeding on a poor host with little nutritional value or with competition may provide the necessary payoff in fitness to make up for risks involved in searching for hosts.

We must also consider the possibility that in the event that a moth fails to oviposit on the host plants, larvae may have to find the plants. In addition, herbivore movement is a constraint in the organization of plant-herbivore systems. If movement is limited, there will be less opportunity for herbivores to respond to variation in host plant quality. The interplay of movement and host selection can even influence the degree to which herbivore communities appear to be competitively organized (Kareiva, 1982). On an evolutionary time scale, natural selection may operate on the mobility of phytophagous insects as a means of improving a species' ability to contend with heterogeneous host quality (Kareiva, 1982). In this respect, mobility of larvae would enable species to cope better with host quality variation,

and consequently be part of the selection and evolutionary context.

A disadvantage of a capability for larval searching to find suitable plants is that in such species, selection may not consistently favor females that oviposit on a certain plant species, unless that species of plant is particularly favorable for the survival of eggs and early instar larvae or unless those hosts are clumped, allowing for larvae to move from one plant to another (Thompson and Pellmyr, 1991). However if moths perform habitat selection instead of plant selection, larval movement would not oppose female choice and the species will be selected favorably.

Assuming that larval displacement to find a host plant is advantageous, polyphagy will be of greater benefit to the immatures. If larvae fall off the host, or host quality declines, or the immature consume all the host leaves, immatures will be more likely to survive if they have wide host tolerances (Mitchell, 1981).

In simulation models, the success of reaching a plant will be correlated with the distance. A negative exponential-type drop off in numbers of animals reaching a site was predicted with increasing distance from the starting point. In addition, percent success declines with increasing mortality and decreased directionality (Kitching, 1971).

The absolute distance that a small caterpillar has to move to cross a surface can differ considerably if the surface is anything other than completely smooth: small

larvae more closely follow the ups and downs of the surface than large caterpillars (Reavey, 1993). Weiss and Murphy (1988) demonstrated this using a fractal model of a rough surface. A 1-mm-long caterpillar would have to travel 15.8 m to cover 1 linear meter, whereas 5-mm caterpillars would travel 8.3 m to cover 1 linear meter. However, larger caterpillars are more vulnerable to enemies. In addition, although larger larvae move faster (Feichtinger and Reavey, 1989), their power and agility do not increase in proportion to their weight (Enders, 1976).

The motility and searching ability of larvae also will influence their distribution on plants in an habitat, which may explain in part the resource concentration hypothesis (Root, 1973). The resource concentration hypothesis suggests that specialized herbivores should be found in the densest patches of their hosts. Since species with this quality (specialists) will be more abundant in a vegetation patch, there is generally more movement on the part of foraging herbivores when food plants or food-plant patches are close together (Kareiva, 1983). The ability to travel exhibited by the specialist *H. subflexa* (monophagous) may well be an adaptation to move between plants or patches of host plants.

Cain (1985), using stochastic simulations, concluded that clumped plants are more difficult for searching insects to locate than are randomly distributed plants. First instar larvae of *P. rapae* had more difficulty finding clumped collard plants than collards spaced at regular intervals,

although total plant densities were identical. Starved larvae and late instar larvae of *P. rapae* tended to find plants more readily than did unstarved or younger larvae (Cain et al., 1985). Rausher (1981a), studying *Battus philenor*, showed that isolated plants do indeed have greater probability than clumped plants of being discovered by dispersing larvae. Changes in the density of the native vegetation did not decrease the efficacy of searching by *B. philenor* larvae. Nevertheless, stems and leaves of non-hosts caused them to change their direction of movement and thus altered search behavior.

Vertical movement in larvae of *H. subflexa*, *T. ni* and *S. exigua* was less than horizontal movement. This difference suggests a higher energy expenditure during climbing in all three species studied. Energy expenditure, however, did not differ in cockroaches running an incline of up to 25° (Herreid et al., 1981).

Large *Lymantria dispar* (L.) larvae climbed at a mean rate of 10.9 cm/min. and small larvae climbed at 6.5 cm/min (Capinera and Barbosa, 1976). In my studies, *H. subflexa*, *T. ni and S. exigua* climbed at 2.9 cm/min, 2.5 cm/min, and 1.1 cm/min, respectively. However, *L. dispar* larvae are approximately seven, eight, and ten times heavier than *H. subflexa, T. ni and S. exigua* larvae, respectively (Capinera and Barbosa, 1976).

The factors (phototaxis or geotaxis) that stimulate the climbing by larvae of the three species are unknown. *T. ni*

exhibited negative phototaxis when on cabbage leaves (Greene and Morrill, 1970; Leppla and Guy, 1980). Rice leafroller, *Cnaphalocrocis medinalis* (Guenée) on non-plant substrates were positively phototactic. When on host plants, the response in this species was reversed (Ramachadran and Khan, 1991). Shields and Wyman (1984) reported that first instar larvae of *Peridroma saucia* are positively phototactic in the laboratory. Starvation increases larval photoresponse in three species of tent caterpillars (*Malacosoma* spp.). However, *Hyphantria textor* Harris became more photonegative when starved (Wellington et al., 1954).

The ability of larvae to climb could determine their success in reaching a preferred part of a host plant. *Spodoptera exigua* larvae move upwards to the top of plants after hatching and feed on the upper foliage layers; this response is governed by negative geotropic and positive phototropic reactions (Smits et al., 1987; Griswold and Trumble, 1985). *Chilo partellus* larvae move from lower and older leaves, where oviposition takes place, to the leaf whorls (Ampofo and Nyangiri, 1986). *Laspeyresia pomonella* (L.) larvae must find apple fruit from the oviposition site, which is usually on leaves near fruits. Searching success depends on the distance the larva is from the fruit at hatching and the complexity of the intervening branches (Jackson, 1979).

An aspect worth pointing out is that movement in generalist feeders would permit a larva to temporarily feed

on a host and, if it is not suitable, the larva could move to another plant. Such behavior would convey advantages over less mobile species. Additionally, they would be able to escape from parasitoids attracted to damaged plants and frass, or from toxic induced secondary plant chemicals released after feeding.

During climbing, larvae of *H. subflexa* and *T. ni* may release a thread of silk to lower themselves. This behavior was not observed in *S. exigua* during the experiments. Wilson (1932) reported that first instar larvae of *S. exigua* release a silk thread with which to be lowered from a plant or to be carried considerable distances by wind. This behavior was not observed during my experiments of vertical movement. *S. exigua* larvae would not release a silk thread when disturbed to lower themselves. However, the absence of this characteristic may have been lost in the selection of the laboratory strain.

It is known that the behavior and capabilities of insects may be subject to selection during the colonization of laboratory-reared insects (Barlett, 1984). In any case, we would expect selection towards less movement by larvae in a laboratory colony. Leppla and Guy (1980) showed that survival and dispersal patterns were different for the laboratory and wild-type strains of *T. ni* larvae. Wild-type strains tended to migrate more than the laboratory strain. In addition, conflicting observations may also be justified

by the existence of "host races" and "biotypes" reported in the entomological literature (Futuyma and Peterson, 1985).

CHAPTER 4
ATTRACTION OF FIRST INSTAR
HELIOTHIS SUBFLEXA (GUENEE), *TRICHOPLUSIA NI* (HÜBNER), AND
SPODOPTERA EXIGUA (HÜBNER) TO HOST-PLANT ODOR

Introduction

Performance of phytophagous insects in host finding has
been considered almost exclusively in relation to adult
sensory capabilities. Host ranges and food preferences of
oligophagous and polyphagous insects have been based on
laboratory experiments or derived from tabulations of species
of plants upon which the insects were found. Little is known
of larval capabilities for searching, finding, and selecting
suitable plants. Although early studies (Brues, 1920;
Dethier, 1941a,b) recognized the chemical attraction of
larvae to plants, they did not consider the potential of host
selection by newly hatched larvae. The basic hypothesis on
the evolution of host selection by phytophagous insects is
that ovipositing females choose plant species that maximize
larval survival and growth, and consequently that larval food
selection is limited (Thompson and Pellmyr, 1991).

Although host chemistry has been considered the major
force in the evolution of diet of plant-feeding insects, it
is being reconsidered due to the poor correlation between
host choice and host suitability (Courtney and Kibota, 1990).

73

However, in this reappraisal the potential of larval host selection as part of the evolution of insect-plant relationships is not considered (Rausher, 1985).

It is believed that basic behavioral patterns involved in food finding are similar in most insects, but that the types of stimuli required to elicit positive responses vary greatly and frequently are species-specific (Hsiao, 1985). Thus, herbivorous insects exhibit extremely variable patterns of response to plant odors. A diverse group of secondary plant chemicals has been found to be important for host plant finding behavior (Hsiao, 1985 and references therein). Restricted feeders (monophagous, oligophagous) tend to respond to specific chemicals from their host. For example, isothiocyanates are attractants and oviposition stimulants for several cruciferous feeders, such as *Pieris brassicae* and *Plutella maculipennis* (Hsiao, 1985).

One of the tools used to study insect host-plant relationships is the olfactory bioassay. Olfactory attractant bioassays are based on the movement of the insect up an odor gradient in an air stream toward an odor source (Kennedy, 1977). This technique has been used successfully in determining attractants of larval stages of insects such as *Dalia antiqua* and *Listroderes obliquus* (Matsumoto, 1970).

Dethier (1976) has suggested that host finding, host recognition, and host acceptance in plant-feeding insects generally are based on mixed olfactory and gustatory sensory information. Additionally, the chemical recognition of host

plants by insects appears to be based on the total mixture of components, although some may dominate the mixture. These in turn form the basis for central integration. The olfactory receptors in the caterpillar antennae are believed to be sixteen bipolar neurons (Dethier, 1941b; Morita and Yamashita, 1961).

It has been mentioned that preferences of plants can be between different species or between individual plants of the same species (Kareiva, 1982). The ability to select a preferred plant would rely partly on individual movement capabilities and mechanisms of recognition (i.e., olfaction).

Host-related behaviors, including larval behavior, of non-pest species of insects like *H. subflexa* (Mitchell and Heath, 1987) have received little attention. However, even with most agricultural pests, studies pertaining to host selection behavior are quite limited (Fitt, 1991). Indirect evidence shows that lepidopterous larvae are capable of movement. For example, Robinson (1978) demonstrated greater dispersal of *Ostrinia nubilalis* neonate larvae from resistant maize hybrids than from susceptible lines.

The objective of this research was to study the orientation capabilities of larval *Heliothis subflexa*, *Trichoplusia ni*, and *Spodoptera exigua*, in response to host and non-host plant odors in the laboratory. This work was in conjunction with studies of movement abilities and survivorship of larvae described in earlier chapters, and the results should permit consideration of the general potential

of first instar larvae of these species for host search and host selection.

Materials And Methods

Newly hatched larvae of the three species were starved for six hours and tested for responses to host plant odor in a four-choice olfactometer developed by Vet et al. (1983). The system consisted of a transparent Plexiglas chamber (Figure 18). The floor is an octagonal sheet of Plexiglas with four long and four short sides (170 x 40 x 11 mm) on which four Plexiglas crescents (90° arc, radius 135 mm, 11 mm high) were glued with cement (Weld-on #16; Industrial Polychemical Service; Gardena, California). The ceiling of the chamber consisted of a Plexiglas sheet of the same shape as the floor, secured to the rest of the chamber by Teflon screws. The chamber was placed on four legs (120 mm). The exposure chamber has a star-like shape. Each point of the star runs into a 5 mm (ID) opening, which conduces to a Teflon tube (5 mm ID). A glass tube was connected to the Teflon tube, to serve as the trap for attracted larvae.

Four defined odor fields are created inside the chamber by pumping purified air (through activated charcoal) through the four arms and sucking air through a hole (17 mm D) in the center of the floor. The airflow in the arms was regulated individually with flow-meters at 200 ml/min (Aalborg Instruments; Monsey, New York; sapphire float). The airflow of the air vacuumed was set at 7 l/min (Aalborg Instruments;

Figure 18. Diagram of the four-choice olfactometer and airflow connections. Four defined odor fields are formed inside the chamber. Air is pulled out through a circular opening on the floor of the chamber.

78

Olfactometer Chamber

Bottom Exit

Vacuum

Airflow

Flowmeter

Vacuum

trap

Airflow

Plant Material

Water

Flowmeters

10- 9- 8- 7- 6- 5- 4- 3- 2- 1-

10- 9- 8- 7- 6- 5- 4- 3- 2- 1-

10- 9- 8- 7- 6- 5- 4- 3- 2- 1-

PUMP
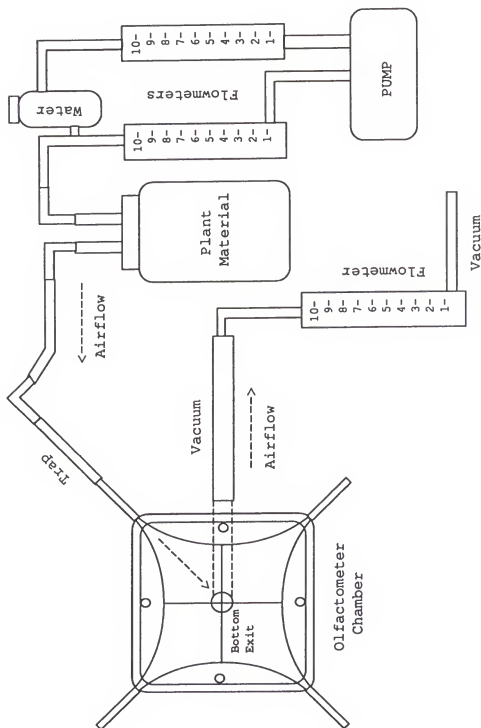
Monsey, New York; carboloy float). The vacuum tube (20 mm, 9 mm ID) that evacuated air from the olfactometer was connected to a house vacuum system by a rubber tube (5 mm ID). The vacuum tube opening was positioned 10 mm under the hole in the floor of the olfactometer chamber to avoid negative pressure disturbances inside the chamber. The air delivered to the chamber was humidified (80% RH) by mixing dry air and air flowing through an air diffusion bottle containing deionized water. Air was filtered through an activated charcoal filter. The odor sources were placed in glass jars (4 l) through which air was flowing carrying the odor. The inside of jar lids was covered with Teflon. Light was provided by overhead fluorescent lamps. Temperature was held at 25°C and relative humidity at 60%. All airflow connections were made with corrugated Teflon tubing. The olfactometer chamber and tubing were washed with hot water and 70% ethanol after every assay. Between assays, the system was ventilated for at least six hours. The position of the arms conducting the test odor was rotated in each assay repetition while maintaining the position of the chamber.

Insects were introduced through the hole in the floor of the chamber using a soft camel hair brush. The airflow in the system was shut off while the insects were introduced into the chamber. Ten insects were used at a time, with a total of eight assay repetitions performed for each experiment. Each assay was conducted for one hour. Response

of insects was considered positive when they entered the
opening leading to the trap. Responding insects were removed
with a hand mini vacuum cleaner (Mini-Vac) when they reached
the trap.

Attraction to a Single Plant Odor (Noncompetitive)

Each of the three species of insects was tested for
responses to odor from host plants presented one at a time in
the olfactometer. Cut plants placed in a 4 l glass jar were
used as the odor source.

Heliothis subflexa larvae were tested for responses both
to foliage and to plants bearing fruit and flowers of
tomatillo (*Physalis ixocarpa* Brot., Solanaceae) and field-
collected *Physalis angulata* L. For each plant species, four
repetitions were performed using only foliage, and four
repetitions were performed using foliage with flowers and
fruit. Ten insects were tested per repetition (n= 40).

Trichoplusia ni was tested against odor from known host
cultivars of cotton (*Gossypium hirsutum*: Malvaceae) and
cabbage (*Brassica oleracea* var. *capitata*, Brassicaceae), and
from two wild plant species on which the performance of
larvae was known, cut-leaved evening primrose (*Oenothera
laciniata*: Onagraceae), and wild lettuce (*Lactuca
graminifolia*: Asteraceae) (Landolt and Lovvorn, 1995 in
preparation). According to Landolt and Lovvorn (1995, in
preparation), wild lettuce is a better host than cotton or
cabbage, while primrose is not a host. Cotton plants were 15

± 5 cm tall and without flowers or fruit, while cabbage
plants were 14 ± 2 cm x 16 ± 2 cm and possessed three rows of
leaves when used. Wild plants were collected from the field
daily between 0900h and 1100h before the experiment and were
kept in a jar of water in the laboratory until used. Cut
greenhouse plants were placed in the glass jar of the
olfactometer one hour before the experiment, with the system
airflow on. Eight repetitions per plant were performed, with
ten insects per repetition (n=80).

   Spodoptera exigua was tested for response to odor from
Amaranthus spinosus L. (Amaranthaceae), a known wild host
(Taylor, 1931). The field-collected plants were collected 5-
6 hours before the experiment and were kept with stems in
water. Plants were rinsed with deionized water and were
dried with a jet of air before testing in the olfactometer.
Eight repetitions per plant were performed, with ten insects
per repetition (n= 80).

   Three of the four fields of the olfactometer during the
assays had only humidified air (80%), while the fourth
contained plant odor. Airflow was set at 200 ml/min for all
four arms.

   Cotton and cabbage were greenhouse-grown. Tomatillo was
grown at the organic garden of the University of Florida from
commercial seeds. Cut-leaved evening primrose, wild lettuce,
pigweed, and *P. angulata* were collected in old fields in
Alachua County, Florida.

Attraction to Multiple Host Odor (Competitive)

T. ni larvae were exposed to odors from wild lettuce, cotton, cabbage, and cut-leaved evening primrose at the same time in the olfactometer. Weight of plant material (40-50 g) was similar for each species in each assay. The purpose of this assay was to test the response of the larvae of *T. ni* to odor from a host and a non-host. Eight repetitions of the assay were performed, with ten insects per repetition (n= 80).

Attraction of *T. ni* to Cotton Extracts

Volatiles from cotton were obtained by collecting the plant odor on an adsorbant (Tenax) for 24 hours. A cotton plant (15 cm) was cut and placed in a glass chamber (4 l). Charcoal-filtered air was pulled through the chamber at an airflow rate of one l/min. Odorants trapped from the airstream onto the Tenax filter were extracted from the adsorbant with methanol and methylene chloride. Doses of cotton volatiles tested were equivalent to the volatiles trapped on Tenax from one plant for 0 (control), 0.5, 1, 3, 10, 30, and 60 min. Each dosage tested contained 200 ul of extract. The extract was placed on a piece of circular filter paper (Qualitative No.2, 4.25 cm, Thomas Scientific, Swedesboro, New Jersey) suspended by a wire in the glass jar of the olfactometer. Air flow was passed through the olfactometer system for 15 minutes before insects were

introduced in the olfactometer chamber. During each assay of this experiment, there were two fields with extract odor, and two controls (solvent alone). Odor and control fields were adjacent and alternated. Two repetitions per dose were performed, with ten insects per repetition (n= 20).

Chi square analyses were performed on the proportion of insects responding to plant odor. The 0.05 probability level was chosen to indicate significance. All statistical analyses were done with JMP-IN™ software (SAS Institute, Cary, North Carolina) in a Macintosh PC.

Results

Attraction to a Single Plant Odor

Heliothis subflexa larvae did not exhibit attraction to host plant odor. Foliage or plants with fruits and flowers of tomatillo and wild *Physalis angulata* elicited very low or non-significant directed movement towards the chamber opening carrying the plant odor. Three larvae entered the controls. Larvae appeared healthy and were active in all assays.

Trichoplusia ni larvae were attracted to wild lettuce, cotton and cabbage. The number of larvae responding to odor from the plants versus the control (three fields) were: cotton 22 vs. 13, cabbage 23 vs. 16, and wild lettuce 32 vs. 13. All the values showed significant attraction to the plant odors (P <0.0001). Cut-leaved evening primrose, a non-host, did not elicit significant attraction in larvae of *T*.

ni (plant odor 8 vs. 9 control) (P= 0.18) (Table 1). The position of the jar containing the test plant did not appear to affect the response of insects. The percentage of non-responding larvae in this experiment comprised between 44% and 79% of the total of larvae tested per plant (n= 80). Non-responding larvae were actively traveling in the chamber.

Larvae of the polyphagous *Spodoptera exigua* were significantly attracted to odor from the wild host *Amaranthus spinosus*. Twenty larvae (25%) were attracted to the host plant odor (P <0.0001). Larvae did not (0%) enter openings delivering humidified air. Non-responding insects exhibited constant movement with very short periods of inactivity.

Attraction to Multiple Plant Odor

Trichoplusia ni ability to select between a host and a non-host plant was demonstrated with this experiment. Larvae were able to differentiate between the known hosts wild lettuce, cotton, and cabbage and a non-host, cut-leaved evening primrose (Chi^2= 7.85, P= 0.049). The expected proportion of responding insects (8.25) to each host plant showed a higher tendency of attraction to cotton (13) and wild lettuce (10) than to cabbage (8) (Table 2). Non-responding larvae comprised 59% of the total number of larvae tested (n= 80) and were actively moving around the olfactometer chamber.

Table 1. Attraction of newly hatched larvae of
Trichoplusia ni to single plant odors in a four-choice
olfactometer. Odor field 1 contained plant odor, the
other three fields contained only humidified air. N= 80
larvae per plant.

Plant	Odor Field		Chi²-Value	P-Value
	1 Plant	2+3+4 Air		
Cotton	22	13	26.8	<0.0001
Cabbage	23	16	26.9	<0.0001
Wild Lettuce	32	13	51.4	<0.0001
Primrose	8	9	4.9	0.18

Table 2. Attraction of *Trichoplusia ni* larvae to
multiple plant odor (competitive test) in a four-choice
olfactometer. n= 80.

	Odor Field				Chi^2 Value	P-Value
	Cotton	Cabbage	Wild Lettuce	Primrose		
No. Larvae Responding	13	8	10	2	7.85	0.049

Attraction of *T. ni* to Cotton Extracts

T. ni larvae were not attracted to cotton extracts using
the four-choice olfactometer. Only two larvae responded to
extracts, one larva responded to the dose of 1 min. plant/200
ul, and one to the dose of 3 min. plant/200 ul. There was
also one larva that entered a control.

Discussion

The significant attraction of first instar *T. ni* and *S.
exigua* larvae to odors from suitable host plants in a four-
choice olfactometer suggests that the chemical composition or
amounts of relevant chemicals in host odors are responsible
for adequate stimulation of sensory organs of the larvae.
This implies that larvae have the potential to select a host
plant based on odor cues.

The ability of larvae to perceive plant material at a
distance would provide mechanisms for successful host plant
finding. The number of plants to be visited before selecting
a host would be fewer than if the insect is required to make
contact with the plant to recognize it as a host. In
addition, when contact stimuli are required the insect has no
method of simultaneous comparison, as may occur using odor
cues (Browne, 1977).

The attraction of *T. ni* larvae to different host plants
may be interpreted as larval attraction in response to either

one common chemical present in several plants or to any of a number of chemicals. Since the larvae are able to differentiate between a host and a non-host plant, there must be some specificity in response to plant odors. However, an answer to this question requires further investigation into the chemistry of host plant odors. Something similar occurs with *Bombyx mori*, which is attracted to chemicals present widely in green plants (Watanabe, 1958).

The differential attraction of *T. ni* to hosts and non-host plant species mimics known patterns of host suitability (Landolt and Lovvorn, 1995, in preparation). On the contrary, first instar *Bombyx mori* are attracted equally to leaves from host and non-host plants; consequently, they could not discriminate between acceptable and non-acceptable hosts (Ishikawa et al., 1969). In addition, based on the present olfactometer results, we can assume that *T. ni* larvae are able to compare and choose from a variety of available hosts, at least over a short distance. This does not agree with the hypothesis that foraging in herbivorous insects is a series of take-it-or-leave-it situations (Thorsteinson, 1960). In addition, it is known that discrimination between hosts can be exhibited in absolute or graded responses. There may even be discrimination between different host plant phenotypes, as demonstrated by Mitchell (1977) for *Pieris brassicae*. *Chilo partellus* larvae also showed differential attraction towards susceptible sorghum cultivars (Saxena, 1990). These observations suggest that caterpillars are

capable of differential attraction to plants of the same
species with relatively minor differences in chemical
characteristics. My results suggest that moderately
polyphagous species such as *T. ni* are not restricted in their
chemosensory requirements as indicated by Hsiao (1985).
Thus, they may not be extremely sensitive to negative
stimuli, and would not require the full complement of
chemicals to respond to a host plant as stated by Hsiao
(1985).

The differential response to four different hosts by *T.
ni* larvae may be related to the presence of similar chemicals
in the plants. For example, general mixtures of green leaf
volatiles have been demonstrated as olfactory attractants for
several insect species (Hsiao, 1985 and references therein).
The difference in rates of attraction observed between assays
testing one versus multiple plant choices may be due to
reduced reaction to host-plant odors when mixed with non-host
volatiles (Nottingham, 1987). However this was not directly
tested. Whether attractants or repellents are responsible
for the differential responses observed in *T. ni* remains to
be investigated.

The negative results obtained in testing for *T. ni*
larval attraction to cotton extracts may be due to several
factors. Extraction procedures may have destroyed the active
odor components, altered ratios of attractants and
repellents, or may have failed to extract attractants from
the cotton foliage. The possibility also exists that the

insect is responding to multicomponent chemical systems rather than to individual components, for which an appropriate ratio and release rate are necessary.

The high attraction response to host odor showed by the larvae of the polyphagous *S. exigua* may indicate a high specificity when responding to positive stimuli from that host or a high tolerance to negative stimuli (Hsiao, 1985). It is known that some polyphagous insects are stimulated by specific plant allelochemicals (Städler, 1992).

Although the mechanisms of olfaction at a distance are still under investigation, it has been demonstrated that when only chemical information is available, walking insects can locate an odor source by temporal and instantaneous comparisons (Bell, 1984). Apparently, only antennal receptors are responsible for perceiving the odors of plant leaves from a certain distance (Ishikawa et al., 1969).

The observed complete lack of attraction of *Heliothis subflexa* larvae to host plant foliage, flowers and fruit indicates an inability of larvae of this species to recognize host plants at a distance. This may be due to high specificity of females laying the eggs on the host plant. The fact that the species is monophagous indicates that adult selection predominates, as predicted by Mitchell (1981). Apparently, the host-selection behavior of *H. subflexa* larvae is similar to that of other caterpillars. They may wander randomly and cannot identify the host plant at a distance, but only after they contact and taste it (Dethier, 1959;

Rausher, 1981a). On the contrary, larvae of *Psilae rosae*, with a restricted host range (Umbelliferae), orient to both primary and secondary plant metabolites (Jones and Coaker, 1977).

It is also necessary to stress that due to the differences in behavior and physiology, a bioassay developed for one species may not work for another species (Hsiao, 1985). A negative bioassay result may not indicate a lack of ability by larvae to respond to plant odors, but a lack of knowledge of what conditions are required for such a response. In addition, it has been suggested that different species evolve different receptor characteristics. Even species sharing the same food plant respond differently to the same stimuli (Schoonhoven, 1973).

Nevertheless, the motility exhibited by larvae of *H. subflexa* suggests the potential for interplant movement to find unoccupied fruit. Interplant movement also appears likely because larvae are competitive and cannibalistic, resulting in one larva per fruit in the field. Other locomotory changes, such as orthokinesis and klinokinesis, may be possible responses by *H. subflexa* larvae towards host plant odors.

Attraction of caterpillars to odor of plants has been demonstrated previously; however, the mechanisms involved were not determined. For example, it has been previously demonstrated that larvae of *T. ni* (Khan et al., 1987), *Danaus plexippus* and *Papilio* spp. (Dethier, 1937, 1941a) are

attracted to host plant volatiles. In addition, Saxena and
Prabha (1975) reported that *Papilio demoleus* fifth-instar
larvae were attracted to individual chemicals from citrus
leaves in a T-tube olfactometer. The response of the larvae
of *T. ni* and *S. exigua* to plant odor in the olfactometer
allowed the insects to maneuver in an horizontal airstream
that carries the odor. This, according to Kennedy (1977),
would allow the insect to use positive anemotaxis for
guidance toward the odor source. Additionally, the steep
odor gradients at the interface between two airstreams would
permit chemotactic and chemoklinokinetic responses. However,
such steep odor gradients are unlikely in the field (Kennedy,
1977). Nevertheless, the demonstration of anemotaxis and
chemotaxis in first instar larvae of *T. ni* and *S. exigua*
shows some potential for host finding and host selection by
newly hatched larvae.

    The attraction of larvae of *T. ni* and *S. exigua* at a
distance might be possible, since they respond
anemotactically and chemotactically to host plant odors.
Although the olfaction of caterpillars is very refined
(Dethier, 1937; Schoonhoven and Dethier, 1966; Dethier and
Schoonhoven, 1969; Saxena and Prabha, 1975), it is considered
effective only at short range. Saxena et al. (1977) reported
responses up to 1 cm, but more typically up to 5 mm (Dethier,
1937, 1941a; Gupta and Thorsteinson, 1960; Chew, 1977; Cain
et al., 1985). However, it has been shown that sensillae of
Lepidoptera larvae have a higher discriminating capacity than

those of adults (Schoonhoven, 1973; Dethier, 1980). In addition, there is indirect evidence that Lepidoptera larvae are capable of host finding at longer distances. For example, *Pieris rapae* larvae are capable of detecting a preferred food plant from a distance of at least 120 mm, and wind is important for the detection of a food plant (Cain et al., 1985). In the field, *Mellitaea harrisii* larvae became distributed unevenly on host plants after dispersal, which suggests that olfaction-based orientation may operate (Dethier, 1959).

To understand the host selection processes for a phytophagous species, we must consider both the role of the adult and the role of the larva. Host detection at a distance and movement abilities of first instar larvae of *T. ni* and *S. exigua* suggest that larvae could increase their performance. Host selection by larvae along with host selection by the adult female could be considered as two levels influencing the female's fitness. It has been suggested that if larvae move to find suitable plants, selection may not consistently favor females that oviposit on a certain plant species. To obtain an advantage from larval host selection, the plant should be particularly favorable for the survival of eggs and early instar larvae, or host plants should be clumped, allowing for larvae to move from one plant to another (Thompson and Pellmyr, 1991).

There are three environmental levels considered in food finding: the habitat, the food plant patch, and the food item

(Hassell and Southwood, 1978). Moths with larvae like *T. ni* and *S. exigua* that are capable of host finding may need to find only the food plant patch or its habitat. Additionally, due to higher discriminatory olfaction than the adults, larvae would be able to make choices and the result would be a range of greater larval tolerance than adult tolerances to host plants (Mitchell, 1981).

The larval ability to find and select a host would have consequences in the quality of the host chosen, as a result of trade-offs while finding the host. If a caterpillar can search long distances with low risk and low cost, it may be able to be selective. However, if larval movement is restricted, the host specificity (threshold) may decline and the larva would accept a host of lower quality. If motility is low and the costs and risks are high during search, selection would favor individuals that are less specific when choosing a host (Schultz, 1983). As search distance or time increases, risk increases, and the probability of surviving to reach a host decreases. Thus, the threshold of quality required for plant acceptance should decrease with time and distance traveled. Vegetation texture would also affect herbivore mobility by influencing herbivore foraging decisions. For example, there is generally more movement of herbivores when food plants or food plant patches are close together (Kareiva, 1982).

Thus, the potential demonstrated here for first instar larvae of *T. ni* and *S. exigua* to find host plants by

responding to odor cues from the plant, should serve as a basis to include larval orientation as a complement or a component of host finding and host selection by Lepidoptera species. In addition, previous experiences may influence plant choices made by larvae (Hovanitz and Chang, 1962; Hovanitz, 1969).

The use of an olfactometer in the laboratory to study anemotactic responses of small crawling insects has several advantages over other approaches. One of them is the ability to simulate appropriate conditions of temperature, light, humidity and wind speed required for responsiveness of insects. One of the inherent advantages of the four-choice olfactometer design adapted here is that the insect can move freely among four different odor fields. In addition, the use of an olfactometer for caterpillars provides the opportunity to make observations of responses and differentiate between kineses and taxes and between attractant and repellent responses (Hsiao, 1985). Another approach for testing olfactory stimuli for attraction responses is to use traps in an open-field situation (Hsiao, 1985). It is believed that positive responses in olfactometers cannot reliably indicate long range attraction as envisaged in field trapping tests (Kennedy, 1965).

Furthermore, data regarding the quantities of volatiles released from undamaged plants in the field are limited. Finch (1978) estimated that a single rape plant (*Brassica napus*) releases 7 ug of isothiocyanates per day. Städler

(1992) considered that highly specific chemosensory receptors are needed to detect the low concentrations of volatiles from undamaged plants. Thus, it is difficult to decide whether the data obtained in an olfactometer are an indication of long range attraction to host plant odor. The threshold of olfaction reported for caterpillars of *Euchaetias egle* was very high (55 mg/liter); consequently, olfaction has been considered only as a short range sense for larval interaction with plants (Dethier, 1937). This does not agree with the results obtained here with *T. ni* and *S. exigua*, and with other studies (Saxena and Prabha, 1975). At the same time, it has been pointed out that olfaction is similarly acute in monophagous, oligophagous and polyphagous species (Schoonhoven, 1973; Dethier, 1980).

The possibility of obtaining misleading results when working with insect cultures is always present, since selection can influence the insect behavior. Although it is been shown that there are differences between wild insects and insects reared in long-standing cultures (Leppla and Guy, 1980), we expect selection for a loss of ability to orient to host plants. Because insects are reared on diet, we might expect loss of the sensory ability to detect plant odors. However, larval response to host plant odor of *T. ni* and *S. exigua* maintained in culture for 16 years, has not been eliminated.

The host plant compounds that are responsible for the attraction of *T. ni* larvae to cotton, wild lettuce, and

cabbage, and of *S. exigua* larvae to pigweed, are unknown. Knowledge of compounds eliciting this behavior would provide information useful in the breeding of crop plants for resistance to pest insects by decreasing their attractiveness.

A reconsideration of locomotion, sensory physiology, and foraging behavior related to food search behavior by larvae is necessary. Moreover, we should point out that the process of locating and accepting a host plant is variable within and among populations (Papaj and Rausher, 1983). In addition, heterogeneity of plant species, including genetic variation, should be considered. It is important to stress that host selection, as any other biological process, is dynamic and evolving continuously.

The results obtained are valuable to theoretical and applied ecology, such as insect pest management strategies and certainly to the understanding of insect-plant relationships. The knowledge of differential host attraction to host odors, combined with other biological aspects (i.e., within-plant movement) opens the possibility of bait and trap development for immatures (Story and Keaster, 1983; Whitford and Showers, 1984).

The study of the potential of first instar larvae of Lepidoptera to find and select a host by responding to odor cues, and their ability to move considerable distances, will enable us to better understand host finding and host selection by lepidopterous insects. Additionally, the

different diet breadths of the species studied permitted comparisons of the motility of the three species and its ecological implications.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Hatching of *Trichoplusia ni* and *Spodoptera exigua* did not show a diel pattern. Hatching in these insects does not indicate any circadian gate or circadian control over larval eclosion from the egg.

Weight of fertilized eggs and first instar larvae was correlated in all three species. Eggs weighted 77.8 ± 2.7 μg (SE) for *H. subflexa*, 65.6 ± 3.2 μg (SE) for *T. ni* and 48.9 ± 2.9 μg (SE) for *S. exigua*. Larvae of *Heliothis subflexa* were the heaviest, 57.8 ± 2.7 μg (SE), *T. ni* larvae weighed a mean of 48.9 ± 2.4 μg (SE), and *S. exigua* larvae weighed a mean of 32.2 ± 2.4 μg (SE). The maximum lifespan of starved *H. subflexa* larvae was 40 hours; for *T. ni* and *S. exigua*, it was 36 hours. In general, lifespan was correlated with the size of the larvae: species with larger larvae lived longer.

Horizontal movement was greater than vertical movement in all three species of larvae. *Trichoplusia ni* overall traveled longer distances in an horizontal arena (232.3 ± 8.4 (SE) cm/h) than *H. subflexa* (214.1 ± 11.3 (SE) cm/h) and *S. exigua* (141.1 ± 10 (SE) cm/h). In a vertical movement situation, *H. subflexa* traveled longer distances (173.9 ± 5.2 (SE) cm/h) than *T. ni* (152.2 ± 6.1 (SE) cm/h) and *S. exigua* (67.7 ± 4 (SE) cm/h). This result suggests that *H. subflexa*

are better climbers than the other two species. The horizontal and vertical distances traveled were correlated with the size of the larvae. The lifespan of the larvae appeared to be reduced as result of more extensive larval movement.

Overall, *T. ni* and *S. exigua* exhibited a higher rate of turning than *H. subflexa* during horizontal movement. This result suggests that *H. subflexa* larvae travel in a straighter pattern than the other two species of larvae. Rate of turning in *T. ni* gradually increased with time of larval starvation. *H. subflexa* and *S. exigua* larvae did not exhibit a change in the rate of turning as they starved. The three species exhibited a tendency to turn more to either right or left. *H. subflexa* and *S. exigua* turned more to the right, and *T. ni* turned more to the left. The left-handedness when turning in *T. ni* increased as time of starvation increased.

T. ni and *S. exigua* first instar larvae were attracted to host plant foliage odors in assays performed in a four-choice olfactometer. In competitive multiple-choice tests, *T. ni* was capable of choosing a host from a non-host through odor cues. In addition, *T. ni* exhibited graded responses to different host plants. The behavioral responses involved in the response to the odors are likely anemotaxis and chemotaxis. *H. subflexa* did not exhibit attraction to odor from host plant foliage, flowers and fruit, which suggests an

inability of larvae of this species to recognize host plants at a distance.

The combined abilities of motility and host attraction to plant odors of the larvae of the two species of moths, *T. ni* and *S. exigua*, suggest an active role of host searching, host finding and host selection by newly hatched caterpillars.

The applicability of using an olfactometer with small larvae offers the possibility of studying larval behavior in relation to movement patterns (kinesis) in response to stimuli. It would also allow further study of insect-plant relationships of value in both theoretical and applied ecology.

Olfaction of Lepidoptera larvae is considered highly refined (Dethier, 1937; Dethier and Schoonhoven, 1969; Schoonhoven and Dethier, 1966), but it is considered to be effective only at short range (Dethier, 1937, 1941a,b; Chew 1977; Gupta and Thorsteinson, 1960; Cain et al., 1985). Consequently, host plant selection by newly hatched larvae has not been investigated. Also, due to the relatively small size of first instar caterpillars, their motility is thought to be very limited. Thus, study of the capabilities for host finding by Lepidoptera larvae has been neglected.

In this context, a better knowledge of host finding, host location, and host selection by newly hatched larvae would help us to better understand insect-plant relationships

as well as related subjects in theoretical and applied insect ecology.

The results obtained in this research indicate that first instar larvae of *Trichoplusia ni* and *S. exigua* have a considerable ability for horizontal and vertical movement (Chapter 2), and are attracted to host plant odor through anemotactic and chemotactic responses in an olfactometer (Chapter 4). In addition, they can survive starvation while walking for up to 24 and 30 hours, respectively. Thus, these insects may be able to search, locate, and select a new host plant through locomotion and in response to olfactory cues. Additionally, the attraction responses of *T. ni* to host plants in competitive olfactometer tests might suggest a graded selection of host plants mediated through plant odor.

Heliothis subflexa also showed a great ability to walk and climb, but it was unable to orient to host plant odor in an olfactometer. We might conclude that host plant recognition by odor cues by the three species of larvae studied is correlated with oviposition specificity of the adult female.

The ability for extended movement apparently is correlated with the size of the larvae. The specificity of oviposition and diet breadth in the three species studied are not correlated with respective capability of movement. *H. subflexa* (monophagous) exhibited a high rate of movement, while *T. ni* and *S. exigua* (polyphagous) exhibited similar or lower rates of movement.

The amount of movement in all three species depends probably on energy reserves of the newly hatched larvae. The rate of vertical and horizontal movement decreased as the larvae starved. During the second or third hour of observation (larvae 6 and 12 hours old), there is a peak of movement, which indicates an increase of energy expenditure, as reported for other insect species (Green, 1964; Bell et al., 1985).

The rate of turning during horizontal movement suggests that *H. subflexa* travels in a straighter pattern than *T. ni* and *S. exigua*. This pattern would permit *H. subflexa* to travel farther than the other two species, walking at an equal rate of movement. *T. ni* showed a gradual increase of rate of turning as starvation time increased. Thus, *T. ni* movement can be classified as extensive searching (ranging) at the beginning of starvation and then becoming local searching (intensive searching) as starvation time increases. The unchanged rate of turning during starvation displayed by H. subflexa and *S. exigua* has been observed in other insect larvae (Jones, 1977; Bond, 1980; Carter and Dixon, 1982). Thus, we may conclude that searching behavior of starved insects is variable and there is not an all-inclusive model even for insects in the same family.

The three species displayed a tendency to turn to the right or left. *H. subflexa* and *S. exigua* were right-handed, while *T. ni* was left-handed. This tendency in the direction of turning apparently appears as a result of increased time

of starvation, and it has also been reported in apterous *Drosophila melanogaster* (Bell et al., 1985). The internal or external factors determining this kind of behavior are unknown. One may speculate that circling behavior in a less-active larva might be useful in finding other host plants within a food plant patch, as opposed to walking straight and perhaps out of the patch.

Additional studies of Lepidoptera larval behavior and orientation to host plant odor using an olfactometer would doubtless prove fruitful, using conditions similar to those used during this research, which are necessary for responses of *T. ni* and *S. exigua* to host plants. Such work may permit more precise ranking of the acceptability of host plant species to larvae, and lead to the development of attractants and baits for larvae of pest species.

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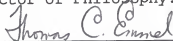Zimmerman, M. (1981). Optimal foraging, plant density and the marginal value theorem. Oecologia, 41, 148-153.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Associate Professor of
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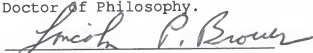
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December, 1995

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